The American Journal of Human Genetics

Vol. 13 No. 2 *June 1961*

The D Trisomy Syndrome and XO Gonadal Dysgenesis in Two Sisters*

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INTRODUCTION

RECENTLY a first case of trisomy for a chromosome of the D group (in the classification by Patau 1960; identical with group 13–15 of the Denver system: Am. J. Human Genet. *12*: 384–388 (1960)) was described by Patau, Smith, Therman, Inhorn, and Wagner (1960). In an addendum a second case of trisomy for evidently the same chromosome was briefly announced. This second D trisomic is case 20 of the present communication. Remarkably enough, a sister of this patient was found to be affected with XO gonadal dysgenesis (Turner's syndrome); she will henceforth be referred to as case 42.

TISSUE CULTURE AND CYTOLOGICAL METHODS

Except for some earlier results obtained by means of short-term cultures of bone marrow (for the technique followed see Patau *et al.*, 1960), the chromosome studies were based on tissue cultures derived either from skin or from bone marrow. The tissue culture techniques were largely those described by Tjio and Puck (1958a). The following remarks pertain not only to the present investigation but also to other work which will be reported elsewhere.

Fresh biopsy specimens were introduced into stoppered tubes of culture medium in which they sometimes had to remain as long as 12 hours, occasionally even as long as 1½ days, before further manipulation. In the case of bone marrow, the specimen, ordinarily about 0.5 ml in volume, was distributed among four 60 mm Petri dishes and placed in the CO_2 incubator. Usually, after two days clearings became visible in the layers of red cells. Apparently all cells in these clearings were fusiform, much larger than hematopoietic cells, often associated with fatty droplets, and with a relatively high mitotic rate. Because of the similarly fusiform appearance of the cells in the cultures finally used for chromosome analysis, it is questionable whether these cultures had descended from blood-forming elements of the bone marrow. The medium in marrow-derived cultures was replaced every third day after the first week. The first subculture was usually started three to four weeks after biopsy. Cover-slip preparations

Received January 3, 1961.

* This investigation was supported by Grant C-3313 from the U.S. Public Health Service and Grant T-167 from the American Cancer Society, Inc.

were made one week later at the second subculturing or, if necessary, from later subcultures.

Each skin biopsy specimen was minced but not trypsinized, and placed in a single 60 mm Petri dish in the CO_2 incubator. Within a few days small fragments of tissue attached to the glass. After one or two weeks they were surrounded by areas of outgrowth. After the second week the medium was renewed every third day. When the cultures contained 10^5 or more cells, *i.e.* usually during the fourth week after biopsy, the first subcultures were started. Cover-slip preparations were sometimes made at this time but usually at the second or, occasionally, at a later subculturing. In every case the cultures consisted of fusiform cells.

The over-all success in establishing cultures for chromosome analysis has been close to 90 per cent for skin specimens and 70 per cent for marrow specimens.

Cells grown on cover slips were fixed as recommended by Tjio and Puck (1958a), but they were not permitted to dry. Instead, the slides were preserved until use in 70 per cent alcohol in the cold. After staining with acetic-orcein or Feulgen reinforced by orcein the preparations were made semi-permanent by framing with Kroenig cement. Individual cells were squashed as needed under microscopic control. The chromosomes were counted by a method (*cf.* Patau *et al.* 1960) that precludes errors arising from subconscious bias.

PARENTS AND SIBS

Both parents are Caucasian; their medical history is unremarkable and there are no known cases of congenital anomaly or mental retardation among their relatives. The parents do not seem to be related to the previously described D trisomic. At the time of conception of the first child, case 42, the mother was 22 years and the father 27 years old. The next two children, also girls, are described as normal. One of them died in an accident at two years of age, the other is now six years old. When the fourth and last child, case 20, was conceived, the maternal age was 35 years. There is no record of abortions.

CASE 42 (XO GONADAL DYSGENESIS)

The patient was 14 years, six months of age when examined by one of us (D.W.S.). She appeared as a typical case of XO gonadal dysgenesis, displaying shortness of stature, posterior webbing of the neck, slight puffiness over the dorsum of the fingers and toes, lack of estrogen effects, and other minor anomalies. Buccal smears were chromatin-negative.

For the cytological analysis a skin tissue culture was available. The chromosome number was found to be 45 (table 1). A detailed analysis was possible in most of the scored cells. There were always four G chromosomes of the kind found in normal females (Fig. 1). Evidently there was no Y chromosome. The presence of only 15 C chromosomes, in conjunction with the buccal smear findings, demonstrated the presence of only one X chromosome. The chromosomes of the autosome groups A, B, D, E, and F appeared normal in number and shape. The cytological finding, a complement as first described by Ford, Jones, Polani, DeAlmeida, and Briggs (1959), thus confirmed the clinical diagnosis of XO gonadal dysgenesis.

TABLE 1. CHROMOSOME COUNTS, NEAR-DIPLOID CELLS ONLY

Patient	Material		≤43	44	45	46	47	48	≥49	Total
	Source	Method								
Case 42	skin	tissue culture	—	1*	19	—	—	—	—	20
Case 20	bone marrow	short-term culture	—	—	—	—	6	—	—	6
	" " (diff. loc.)	tissue culture	—	—	—	—	4	—	—	4
	skin	" "	—	—	—	—	14	1†	—	15
	Total		—	—	—	—	24	1	—	25

* Cell appears unbroken, count reliable.
† Counting error possible.

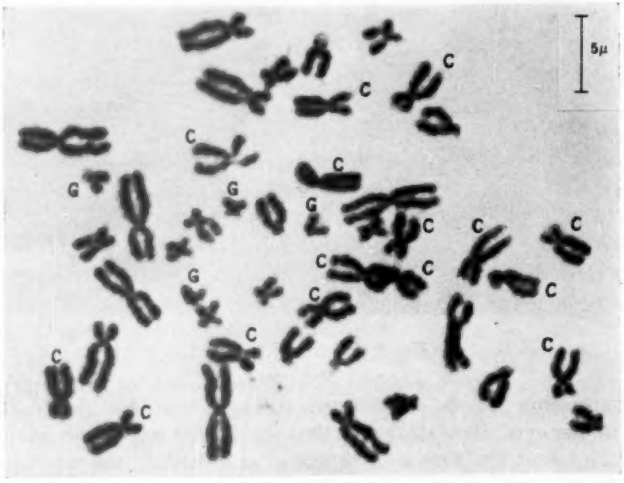

FIG. 1. The 45 chromosomes of case 42. Note 15 C chromosomes and four G chromosomes: XO gonadal dysgenesis. Tissue culture. Feulgen, orcein.

CASE 20 (D TRISOMY SYNDROME)

The patient, a female infant, was born in November, 1958, and is, after two years, still alive. Her mother had a "bad case of flu" with fever and chills during the first trimester of the full-term pregnancy. At birth the patient weighed 7 lb. 7 oz. During her first two days she showed respiratory distress and cyanosis but, as far as is known, not thereafter. Over the posterior fontanelle area of the skull, the skin was absent with the effect that this area appeared "raw and meat-like". This healed spontaneously within three weeks. During her first month the patient was inactive and she seldom cried. When she was nine months old she could, in the prone position, hold up her head, but was not able to sit alone. The parents

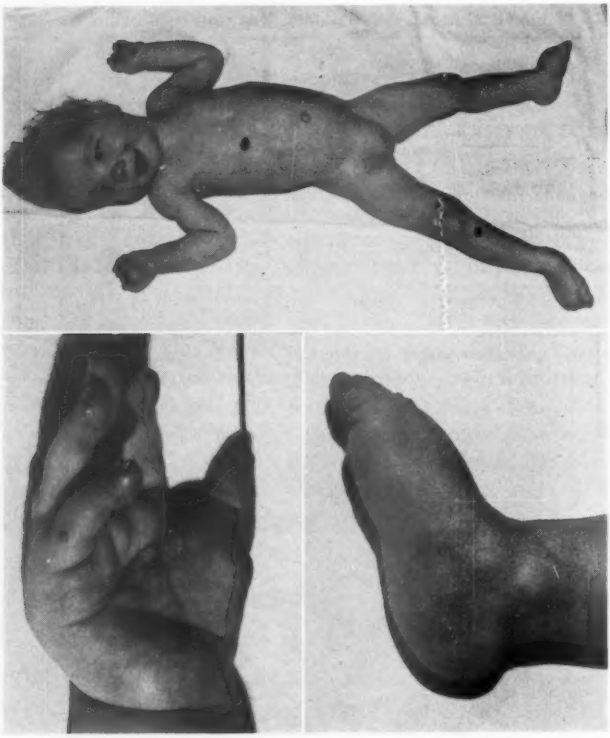

FIG. 2. D trisomic (case 20).

had the impression that she could distinguish light from dark, but did not follow a light with her eyes; they also noted that she did not respond to sound.

When the infant was first seen by one of us (D.W.S.) she was 13 months of age (Fig. 2). Her weight, 18 lb., was 4 lb. under the average for her length of 31.0 in. The head circumference was 17.2 in.

The patient reacted to a strong light by blinking but did not follow moving objects with her relatively small eyes. Each iris had an inferior-medial coloboma, the back of each lens was partially covered by a vascularized white membrane, there were abnormally few retinal vessels, and the optic discs were large, pale, and vertically ovaloid with undercut medial margins.

The patient showed no response to a loud sound, she did not respond even to a slapping of the bed that visibly shook her. The auricles were slightly low-set. The palate had a 2.0 cm long posterior cleft. The frenulum extended to the tip of the tongue, which was slightly cleft. Eight teeth were present and appeared normal.

Both hands had horizontal palmar creases and relatively slim terminal phal-

anges with unusually convex fingernails. The thumbs could be bent backward to an abnormal degree at their rather narrow base, two small "clicks" being sometimes noticeable. This condition of the thumbs resembled that described for the first case of D trisomy (Patau *et al.*, 1960) as "trigger thumb", except that the thumbs of the present patient occasionally assumed the backward bend spontaneously. We have since learned that the term "trigger thumb" has been used by others for a different condition. For this reason, we shall henceforth call the anomaly of the thumb as observed in both D trisomics "retroflexibility" of the thumb.

At the ulnar side of the left fifth finger a 2 mm wide nodule of skin was present. The feet were maintained in a position of moderate downward flexion and had a dorsal cover of non-pitting soft tissue. Their soles were flat and they had posterior projections (1.0 cm) of the heels, an anomaly known as rocker bottom feet.

Raised cherry-red hemangiomata, ranging in diameter from 1 to 3 cm, were present on the trunk, left upper arm, and on the right lower leg.

Judged by her performance, the mental development was below the two-months level. She smiled, but otherwise showed little spontaneous activity and tended to lie on her back with legs extended and arms partially flexed. An occasionally occurring sudden extension of the extremities resembled a myoclonic jerk. The infant was unable to support herself in the sitting position. On passive movement of the extremities, particularly the lower, the muscles were found to be moderately hypotonic. The deep tendon reflexes appeared to be normal.

Roentgenograms disclosed a spina bifida posterior of the first cervical vertebra. The heart was normal in size and contour (blood pressure: 105/70). An electroencephalogram, which showed frequencies of 4 to 8 cycles per second, was interpreted as normal. Further studies, including a pyelogram and laboratory tests of blood and urine, revealed no abnormality.

The patient was subsequently admitted to the Northern Wisconsin Colony and Training School for Retarded Children where she was last seen by us when she was 19 months old. She then followed a moving hand with her eyes but seemed still unable to roll over or sit without support.

For the cytological analysis three different samples were available (table 1). The basic chromosome number of the patient is 47, the extra chromosome belonging to the D group (Figs. 3 and 4). A detailed analysis of all suitable cells revealed that the chromosomes of all groups other than the D group are those of a normal female complement, not only in regard to number but also to chromosome morphology as far as this can be ascertained in favorable mitoses. Buccal smears were chromatin-positive.

It remains impossible to state to which of the three pairs of the D group the extra chromosome belongs (*cf.* Patau *et al.* 1960, and Patau 1960). There would be no need to say more were it not for a recent publication (Hayward and Bower 1960) in which our first case of D trisomy is referred to with the additional, and to the present authors surprising, information that the "trisomic chromosome" is No. 15. No reason for this identification is given, but as the authors use the Denver "standard system" in which No. 15 is defined only by the sentence "no

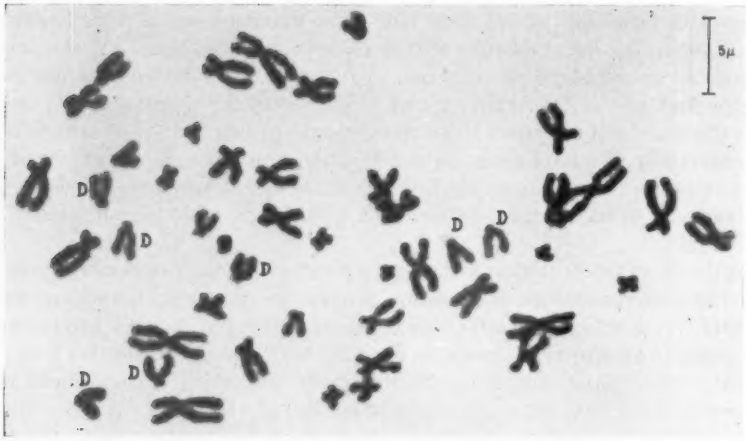

FIG. 3. The 47 chromosomes of case 20. Note seven D chromosomes. Tissue culture. Feulgen, orcein.

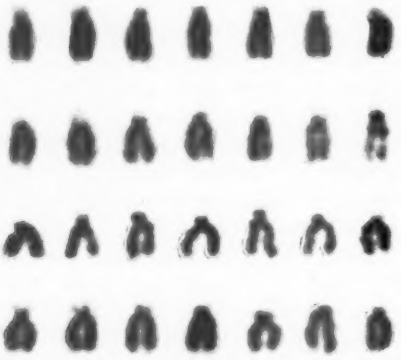

FIG. 4. The seven D chromosomes from four cells of case 20. Tissue culture. Feulgen, orcein. ×3040.

satellite has been detected on chromosome 15", it would seem that Hayward and Bower assume the extra chromosome to have no satellite. To this we offer the following comment.

The satellite of any human satellite chromosome is more often than not unrecognizable, either because its position relative to the short arm that carries it precludes it from being seen as a distinct body (and nothing less justifies speaking of a satellite) or, possibly, because it was torn off during the preparation. To realize the difficulty one needs only to remember that all authors who first described satellites in man assumed that they occur only at one pair of D chromosomes (Tjio and Puck 1958, b; Chu and Giles 1959; Böök, Fraccaro, and Lindsten 1959; Levan and Hsu 1959) although now they seem to agree that two pairs of

TABLE 2. EXPECTED FREQUENCIES (BINOMIAL) OF NUCLEI WITH DIFFERENT NUMBERS OF DISTINGUISHABLE SATELLITES, ASSUMING THAT THE MEAN NUMBER OF VISIBLE SATELLITES PER NUCLEUS IS ONE

D Chromosomes with Visible Satellites, Number per Nucleus	Number of Actually Satellited D Chromosomes:	
	4	6
0	31.64%	33.49%
1	42.19	40.19
2	21.09	20.09
3	4.69	5.36
4	0.39	0.80
5	—	0.06
6	—	0.00
Total	100.00%	99.99%

D chromosomes, No. 13 and No. 14, have satellites (Denver system). We can confirm this, for we have in a few instances seen three D chromosomes with satellites in metaphases from persons with an evidently normal D group. We also have noted that satellites occur at both the smallest and larger D chromosomes. The observations published by other authors do not indicate that they have had much more success than we in demonstrating satellites with any regularity. Neither does it seem that the situation could be greatly improved by any special staining method, as the visibility of orcein stained satellites that are sufficiently set off from the chromosome is satisfactory.

Assume there is in technically favorable mitoses (which implies among other things a low degree of chromosome contraction) a mean number of one recognizably satellited D chromosome per cell, probably a too optimistic estimate. On this basis and under the further assumption of statistical independence of chromosomes in regard to the visibility of satellites table 2 was computed. It will be seen that a very large number of mitoses would have to be scored before any case could be made for the existence of only two pairs of satellited D chromosomes. As long as such a demonstration is lacking, the Denver definition of the individual chromosomes 13–15 is meaningless.

We suspect that this definition will remain meaningless, for there are indications that all D chromosomes have satellites. We have found in a tissue culture of skin from a patient with an apparently normal group of six D chromosomes a prophase nucleus with five clearly satellited D chromosomes. One cell cannot decide the issue but there is some supporting evidence. In blood culture mitoses we have very often found two or more acrocentric chromosomes that were associated by their short arms, *i.e.* the arms that may carry satellites. The association, which has independently also been discovered by Dr. Ferguson-Smith (personal communication), is non-homologous, D and G chromosomes being combined in a random fashion. It seems highly likely that the association is a remnant of an association brought about by nucleolar fusion, the stems of satellites being nucleolar organizers. Polani, Briggs, Ford, Clarke, and Berg (1960) have ably discussed the role that nucleolar fusion in man may play in promoting special types of chromosomal rearrangements and in non-disjunction. Rele-

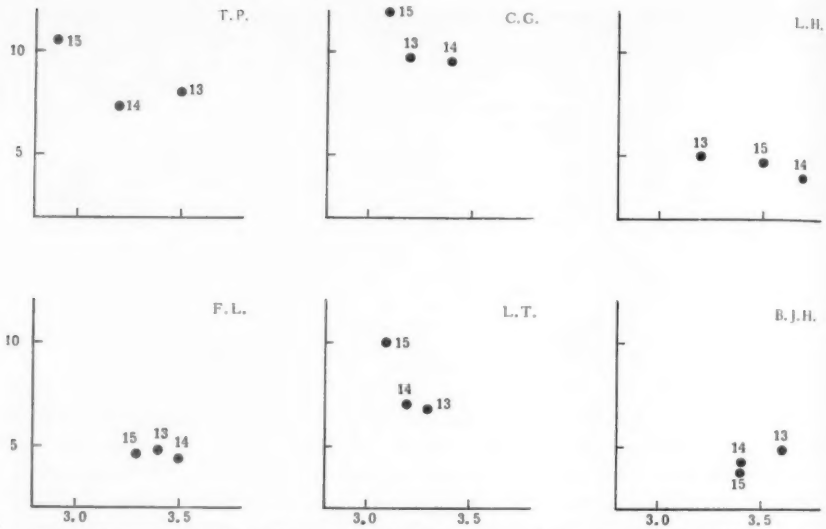

FIG. 5. Karyograms of the D group based on the means of measurements by different authors (identified by initials) as given in Table 2 of the Denver Report. Abscissae: chromosome lengths in percent of haploid total. Ordinates: arm ratios.

vant in the present context is the occurrence, in blood cultures from euploid persons, of mitoses in which five (the highest number found so far) D chromosomes are associated with each other or with G chromosomes. This, again, suggests that all three pairs of D chromosomes have satellites.

If there should be any notion that the individual D chromosomes might be identified, if not by satellites, by their arm lengths, a study of the karyograms (Patau 1960) of Fig. 5 should dispel it. Differences in preparation and measuring technique can cause considerable discrepancies in relative chromosome length and arm ratio between measurements by different authors. This reflects systematic errors that should affect all of the very similar D chromosomes in about the same manner, resulting in shifts of the pattern of points in the karyogram but not in major alterations of the pattern itself. Instead, we find a variety of patterns that clearly contradict each other. Evidently the authors of the Denver Report themselves felt that the relative chromosome length and the arm ratio do not yield a meaningful definition of the chromosomes 13 through 15. As pointed out above, the attempted alternative, definition by satellites, is not practical either. We repeat the previous suggestion (Patau 1960) to discontinue the practice of assigning numbers to chromosomes unless there are well-defined criteria by which they can be identified with assurance. Such criteria do not exist for D chromosomes.

DISCUSSION

The patient described above is the second example known to us of trisomy for a D chromosome, the first having been published by Patau *et al.* (1960) (this

patient, henceforth referred to as case 5, died recently; the autopsy findings will be reported elsewhere). The two cases have in common mental retardation, apparent deafness, myoclonic seizures, an eye defect (case 5: apparent anophthalmia, case 20: microphthalmia, colobomata of the iris, etc.), retroflexibility of the thumbs (called "trigger thumbs" by Patau *et al.* 1960), horizontal palmar creases, cleft palate, and hemangiomata (capillary-case 5, or raised ones-case 20). In addition, both cases have abnormal feet (case 5: polydactyly, case 20: rocker bottom feet). A hare lip and a heart anomaly were found only in case 5. Clearly, the sets of anomalies displayed by both patients are similar enough to be regarded as representatives of the same syndrome.

Hayward and Bower (1960), referring not only to the one trisomy case each that was published by themselves and by Edwards *et al.* (1960) but also to D trisomy as reported by Patau *et al.* (1960), express the opinion that "it has still to be unambiguously demonstrated that autosomal trisomy is the cause rather than an accompanying phenotypic effect expressed at the nuclear level, or a chance association of events". A strange remark. When Patau *et al.* (1960) mentioned in an addendum their second case (20) of D trisomy with a "similar set of congenital anomalies" the association of D trisomy and the syndrome, which obviously are both very rare events, became statistically significant to a degree that amounted to an unambiguous demonstration of this not being a "chance association". There remains the possibility of trisomy being an "accompanying phenotypic effect". How are we to envisage that? Are we, for instance, to assume that the agent responsible for the syndrome also causes non-disjunction not just of any chromosome but of a D chromosome during the first cleavage division? If so, what happens to the cell which is deficient of a D chromosome? It does not seem that the present patient is a mosaic. We must confess that we are unable to think of any remotely plausible alternative to the conclusion that the syndrome is caused by the presence of a specific D chromosome in triplicate, which in turn we take to have resulted from non-disjunction, most likely during the first meiotic division, in one of the parents.

With the methods available to date it is impossible to decide which of the three different D chromosomes is present in triplicate in the two trisomics. We do infer from the similarity of the two patterns of anomalies that in both cases of D trisomy the responsible chromosome is the same. We propose to call it D_1, suggesting that in general chromosomes that are identifiable by the phenotype of trisomics but not with the microscope be denoted by a subscript attached to their group symbol. It is unknown whether trisomics for a D chromosome other than D_1 are viable. So long as none has been observed it will suffice to refer to the "D_1 trisomy syndrome" as the "D syndrome".

More cases will have to be discovered before a satisfactory characterization of the D syndrome can be given. No doubt it will be found to contain anomalies as yet unrecognized or that happened not to manifest themselves in the two cases discussed here. In view of the situation in mongolism, it is also to be expected that some, or even all of the anomalies displayed by these two patients will not always be present. The D syndrome is obviously very rare. We have screened clinically some thousand patients, mostly young and/or low grade, in three state

colonies for the mentally retarded and found only one with the D syndrome, the present case 20, whom we had seen before. In the same sample, there may have been some 250 to 300 mongoloids. Assuming an incidence of mongolism of 1 in 700, this would correspond to a frequency of the D syndrome of 1 in 200,000. Allowing for bias and a very large sampling error, we may safely estimate that the D syndrome is rarer than 1 in 10,000. It should be added that among these thousand patients there were a number who displayed some of the anomalies of the D syndrome without giving the impression of belonging to the same clinical entity as the present two patients. Almost all of these proved to have 46 chromosomes, but evidence has turned up to support the working hypothesis that at least certain "partial D syndromes" are due to the presence of a translocated extra piece of D_1, in other words, that they are duplication effects. The investigation of such cases is being continued.

The word "trisomy" implies the presence of three homologous chromosomes and should not be used when an extra chromosome is suspected to be a translocation chromosome that merely happens to have about the same size and shape as a certain pair of homologues. By the same token, the diagnosis of trisomy of a new type remains necessarily somewhat uncertain until another case is found that combines the same chromosome complement with a similar clinical picture. According to this criterion, only three types of autosomal trisomy can be considered as established to date[1]: mongolism, the D syndrome, and the "E syndrome" described by Smith, Patau, Therman, and Inhorn (1960), to which on clinical grounds the case reported by Edwards, Harnden, Cameron, Crosse, and Wolff (1960) may be added, even though their identification of the extra chromosome as No. 17 conflicts with our own as No. 18, which we consider as definitive (the evidence is being published elsewhere: Patau, Therman, Smith, and DeMars 1961). This trisomy syndrome seems to be somewhat more frequent than the D syndrome, but it is still very rare compared with mongolism. There are two equally plausible mechanisms either of which might explain the low incidence of E and D trisomics. Conceivably, the probability of non-disjunction decreases steeply with increasing chromosome length. Alternatively, trisomy for D_1 and trisomy for chromosome No. 18 might ordinarily be lethal conditions, rendered viable only by specific gene mutations.

It is virtually certain that the co-occurrence in a sibship of two conditions as rare as XO gonadal dysgenesis and the D syndrome is no mere coincidence. An environmental causation of an increased frequency of non-disjunction is conceivable but does not fit too well the circumstance that the two patients were born 13 years apart. Alternatively, and perhaps more likely, one of the parents may

[1] Hayward and Bower (1960) found apparent trisomy in a patient with Sturge-Weber's syndrome. In all cases of this disease that have since been investigated cytologically, by others or ourselves, the chromosome number was 46. However, in one of our cases a translocated extra piece was present. Hayward and Bower's extra chromosome may also have resulted from a translocation. It appears likely that Sturge-Weber's syndrome is generally caused by the presence in triplicate of a translocated segment of an unknown donor chromosome even though this segment may usually be undetectable in the microscope (Patau, Therman, Smith, Inhorn, and Picken 1961: Am. J. Human Genet., in press).

have a genotype that fails to sustain fully all of the processes required for a successful conclusion of meiosis. That there is a genetic control of meiosis could be taken for granted even if it had not been demonstrated in maize, *Drosophila,* and other organisms. Any explanation, except by pure coincidence, of the present case of familial incidence of aneuploidy involving different chromosomes leads one to suspect that zygotes with other abnormal chromosome complements might also have been formed. If these were abnormal enough, abortion might have taken place before the pregnancy was recognized. The birth of two apparently normal children during an interval of thirteen years does not rule out this possibility.

The present case of different types of aneuploidy in two sibs reminds one of the patient with combined mongolism and Klinefelter's syndrome (Ford, Jones, Miller, Mittwoch, Penrose, Ridler, and Shapiro 1959; Harnden, Miller, and Penrose 1960). Here, too, coincidence is extremely unlikely and a genetic causation appears possible, but there is less reason to resort to a genetic explanation than in our case. Individual cells in an otherwise healthy population of gametocytes may show disturbances, of the spindle for instance, that could result in multiple non-disjunction. An increase of the incidence of non-disjunction with maternal age need not reflect an increased probability of non-disjunction in all oocytes. If it does not, if merely the frequency of "disturbed" oocytes were increased, an above-random incidence of multiple non-disjunction in aging mothers (in the British case her age was 40) would result.

SUMMARY

A patient who represents the second case of trisomy for a D chromosome (for the first see Patau *et al.* 1960) is described. Both cases have in common mental retardation, apparent deafness, myoclonic seizures, an eye defect, retroflexibility of the thumbs, horizontal palmar creases, cleft palate, hemangiomata and abnormal feet.

The two cases establish the existence of a "D syndrome" that is caused by the presence in triplicate of a specific D chromosome, even though it is at present not possible to distinguish the latter microscopically from the other two pairs of D chromosomes.

A sister of the present patient is a typical case of XO gonadal dysgenesis. This familial incidence of aneuploidy may be due to a genotype of one of the parents that does not sustain full regularity of meiosis.

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Gm Phenotypes and Genotypes in U. S. Whites and Negroes; in American Indians and Eskimos; in Africans; and in Micronesians*

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FIVE GENETICALLY DETERMINED variants in human serum detected by a hemagglutination inhibition test have been demonstrated in the Gm system (Grubb, 1956; Grubb and Laurell, 1956; Harboe, 1959; Harboe and Lundevall, 1959; Steinberg, Giles, and Stauffer, 1960; and Ropartz, Lenoir, and Rivat, 1961). These factors are called Gm^a, Gm^b, Gm^x, Gm-like, and InV. Each has been shown to be inherited as a dominant or, when appropriately tested, as a codominant.

Population studies have shown that the frequency of Gm^a varies among Caucasians from a low of 40 per cent to a high of about 70 per cent (see Ropartz, 1960 for review; see also Adinolfi *et al.*, 1959 and 1960; and van de Wiel and Dorfmeijer, 1959). The Gm (a+) frequency approaches 100 per cent in the non-white populations which have been tested thus far [Negroes (Moullec, 1959; Podliachouk, 1960; Steinberg, Giles, and Stauffer, 1960; Steinberg, Stauffer, and Boyer, 1960; and Ropartz, 1960), Eskimos (Grubb and Laurell, 1956, and this publication), Indians (Steinberg, Stauffer, and Fudenberg, 1960), Chinese (Kirk, 1958), and Japanese (Yokoyama and Ueno, 1961)]. Negroes are 100 per cent Gm (b+) (Steinberg, Stauffer, and Boyer, 1960, and Ropartz, 1960) as are some other non-white populations (Steinberg, unpublished).

The frequency of Gm^x has not been determined for many populations. It is probably absent in pure Negroes, but is present in whites (Harboe and Lundevall, 1959; Steinberg, Giles, and Stauffer, 1960; and Ropartz, 1960) and other populations (this publication). Gm-like is absent in whites but present in Negroes (Steinberg, Giles, and Stauffer, 1960, and Ropartz, 1960). InV is present in whites and Negroes (Ropartz, Lenoir, and Rivat, 1961).

Only two reports of populations tested for Gm^a, Gm^b, Gm^x, and Gm-like have been published [combined data of Steinberg, Giles, and Stauffer, 1960, and of Steinberg, Stauffer, and Boyer, 1960; and Ropartz, 1960]. In the light of the considerable variability already demonstrated, we decided to test other populations for these factors.

Received January 23, 1961.

* This work was supported in part by PHS grant RG 7214 from the Division of General Medical Sciences.

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MATERIALS AND METHODS

The samples

The populations studied are: U.S. whites and Negroes from the Cleveland area, Eskimos and Indians from Alaska, Negroes from Nigeria, Micronesians from Rongelap Atoll, Marshall Islands, U.S. Trust Territory of the Pacific Islands, Bashi and Pygmies from Africa, and two Indian tribes, Negroes, and Javanese from Surinam, South America.

The Eskimo population samples (northern dialect speaking) are from Wainwright, Alaska. The North American Indian (Athabascan) population samples are from Arctic Village and from Fort Yukon, Alaska. There have been reports on the hemoglobin and haptoglobin patterns (Blumberg, Allison, and Garry, 1959), the urinary amino acid excretion patterns (Allison, Blumberg, and Gartler, 1959), the ability to taste phenylthiocarbamide (Allison and Blumberg, 1959), and on the blood groups of these samples (Corcoran, Allen, Allison, and Blumberg, 1959). The last paper contains a description of the populations.

The Nigerian population samples were collected (Blumberg) from Yorubas in the village of Ilora, near Ibadan in the western region of Nigeria and from Fulanis from various locations in Northern Nigeria. A description of these populations and of their blood groups is in press (Blumberg, Ikin, and Mourant, 1961).

The samples from the Micronesian population were collected on Rongelap Atoll in the Marshall Islands. Blumberg and Gartler (1959) have reported on their β-amino-isobutyric acid excretion patterns.

The samples of the Bashi and the Pygmies were collected by Dr. Arno Motulsky. We are grateful to him for sending us aliquots of these samples. The Bashi are a tribe from the Eastern Province of the Republic of Congo. The samples were collected in Bukavu. The Pygmies are from the Ituri forest.

The Gm^s and Gm-like reactions of the Carib and Oyana Indians, the Djuka Negroes, and the Javanese, all from Surinam, South America, were reported by Steinberg, Stauffer, and Fudenberg (1960).

Rigorously controlled sampling methods were not used for most of these collections. Although there is no evidence that the method of sampling would favor exclusion of one or the other phenotypes, the samples are not truly random. The gene frequencies, therefore, may not be typical for the populations from which the samples were drawn. However, the gene frequencies are probably satisfactory for gross comparisons.

Gm testing

All tests were run at room temperature on microflocculation test slides against O, R^1R^1 red blood cells coated with an incomplete anti-D serum used as indicated in table 1. The cells and the anti-D serum were incubated at 37°C for two hours. The cells were washed four times in normal saline before resuspending them in saline at a 0.3 per cent concentration. The reagents (reagin, the anti-D sera, and the test sera), and their concentrations, used for the several tests are shown in table 1.

TABLE 1. REAGENTS AND THEIR CONCENTRATIONS USED TO TEST FOR Gm^a, Gm^b, Gm^x AND Gm-LIKE

Gm Factor	Anti-D*	Reagin (Agglutinator)		Serum to be Tested Diluted to	
		Name	Dilution		
a	Kim.	Scol.	1/8	1/8 and 1/16	
a	Kim.	Bowers	1/64	1/8	1/16
b	Ham.	Bomb.	1/32	1/8	1/16
x	Ham.	Bowers	1/64	1/16	1/32
like	Warren	Bomb.	1/32	1/16	1/32

* All anti-D sera were used as follows: One drop of anti-D, one drop of packed, washed red blood cells, plus 8 drops of normal saline; the mixture was incubated at 37°C for two hours.

The tests were performed as follows: one drop of diluted test serum was added to one drop of diluted reagin. After this mixture was shaken for 5 minutes, one drop of the 0.3 per cent suspension of the coated red blood cells was added and the mixture was again shaken for 5 minutes. The mixture was incubated in a moist chamber at room temperature for about 50 minutes, shaken for 5 minutes, and then read under a dissecting microscope at 120×.

Sera known to be positive for a given Gm factor and others known to be negative, were tested, as controls, simultaneously with the sera from the populations. All sera were tested for their ability to cause the coated cells to agglutinate, since sera from healthy individuals as well as those from individuals with various diseases may do so (Grubb, 1956; Ropartz *et al.*, 1958; and Ropartz and Lenoir, 1960). Reagin Scol. is from a healthy donor, the others are from patients with rheumatoid arthritis.

THE DATA

The results of the tests of the samples from each of the populations, grouped by phenotype, are shown in table 2. Table 3 presents the frequencies, in per cent, of the various factors in each of the samples.

A word of explanation is required for the Gm^x reaction of the West Africans, and especially the Yoruba. The samples were received in two groups. The first sample consisted of 15 sera. These (as did those received later) when tested for Gm^x gave equivocal results. We could not classify the sera with confidence as either Gm (x+) or Gm (x−). Accordingly, we titrated the 14 samples from the first set for which we had serum left after completing the other tests. Each serum was diluted in saline in two fold dilutions from 1/4 to 1/128 and each of the six dilutions was tested for its ability to inhibit reagin Bowers in the usual test procedure. The results were scored as 0 if inhibition was complete and +, ++, +++, or ++++ for increasing strengths of agglutination. Each sample was assigned a score which was simply the sum of the + signs recorded for the six tests of the serum. From the nature of the scoring system, Gm (x+) sera (inhibitors) would have low scores, and Gm (x−) sera, high scores. Seventeen Gm (x+) sera and 18 Gm (x−) sera were titrated as controls. The results are shown in table 4. The scores of the Yoruba sera fall between those for the Gm (x+) and the Gm (x−) controls. We have no satisfactory explanation for these

TABLE 2. GM PHENOTYPES OF VARIOUS POPULATIONS

Population	No.	+−−−[1]		+−+−		++−−		++−+		−+−−		+++−		++++	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
United States															
White	303	14	4.6	11	3.6	87	28.7	0	—	149	49.2	42	13.9	0	—
Negro	364	8	2.2	1	0.3	240	65.9	98	26.9	7	1.9	7	1.9	3	0.8
Athabascan Indians (Alaska)															
Arctic Village	58	34	58.6	24	41.4	0	—	0	—	0	—	0	—	0	—
Fort Yukon	51	33	64.7	16	31.4	2	3.9	0	—	0	—	0	—	0	—
Total	109	67	61.5	40	36.7	2	1.8	0	—	0	—	0	—	0	—
Eskimos (Alaska)															
Wainwright	50	38	76.0	2	4.0	9	18.0	0	—	0	—	1	2.0	0	—
Africa															
Nigeria Yoruba	35[2]	0	—	0	—	0	—	35	100.0	0	—	0	—	0	—
Nigeria Fulani	35	0	—	0	—	2	5.7	33	94.3	0	—	0	—	0	—
Bashi	37	2	5.4	0	—	22	59.5	13	35.1	0	—	0	—	0	—
Pygmies	120[3]	0	—	0	—	0	—	120	100.0	0	—	0	—	0	—
Micronesia															
Rongelap Atoll	149	4	2.7	0	—	80	53.7	65	43.6	0	—	0	—	0	—
Surinam[4]															
Djuka Negroes	35	0	—	0	—	13	37.1	22	62.9	0	—	0	—	0	—
Javanese	20	0	—	0	—	7	35.0	10	50.0	0	—	3	15.0	0	—
Oyana Indians	15	1	6.7	1	6.7	10	66.7	0	—	0	—	3	20.0	0	—
Carib Indians	19	2	10.5	2	10.5	7	36.8	0	—	0	—	8	42.1	0	—

[1] The first symbol (+ or −) refers to Gm^a, the second Gm^b, the third Gm^x, and the fourth Gm-like.

[2] One of these sera gave an equivocal test for Gm^x and a second was not tested for Gm^a because it agglutinated the coated cells.

[3] An additional serum was not tested for any of the Gm factors because it agglutinated the coated cells, and another which was Gm (a+) could not be tested for the other factors because it agglutinated the coated cells.

[4] Gm^a and Gm-like data previously reported (Steinberg, Stauffer, and Fudenberg, 1960).

data. It is possible that the Yoruba have an intermediate allele for Gm^x. We do not think the high concentration of gammaglobulin in their serum (about twice that of U.S. whites, Fudenberg, unpublished data) can account for this observation, because the Oyana Indians studied by Steinberg, Stauffer, and Fudenberg (1959) have essentially the same concentration of gammaglobulin (Fudenberg, unpublished data) and do not give such reactions for Gm^x.

DISCUSSION

All the non-white populations sampled [except the U.S. Negroes, who have about 30 per cent white ancestry, (Glass and Li, 1953, and Steinberg, Stauffer, and Boyer, 1960)] are 100 per cent Gm (a+) (tables 2 and 3). This is in agreement with previous findings on non-white populations (see Ropartz, 1960 for review). The North American Indians and the Eskimos are low in Gm^b, much lower than any other populations for which there are published reports. [Austral-

TABLE 3. FREQUENCIES (IN PER CENT) OF Gm FACTORS IN VARIOUS POPULATIONS[1]

Popuation	No.	Gm Factor			
		a	b	x	like
United States					
White	303	50.8	91.8	17.5	0
Negro	364	98.1	97.5	3.0	27.7
Athabascan Indians (Alaska)					
Arctic Village	58	100.0	0	41.4	0
Fort Yukon	51	100.0	3.9	31.4	0
Total	109	100.0	1.8	36.7	0
Eskimos (Alaska)					
Wainwright	50	100.0	20.0	6.0	0
Africa					
Yoruba[2]	35	100.0	100.0	0	100.0
Fulani	35	100.0	100.0	0	94.3
Bashi	37	100.0	94.6	0	35.1
Pygmies	120	100.0	100.0	0	100.0
Micronesia					
Rongelap Atoll	149	100.0	97.3	0	43.6
Surinam[2]					
Djuka Negroes	35	100.0	100.0	0	62.9
Javanese	20	100.0	100.0	15.0	50.0
Oyana Indians	15	100.0	86.7	26.7	0
Carib Indians	19	100.0	79.0	52.6	0

[1] See text for further details.
[2] See table 2.

TABLE 4. TITRATION SCORES FOR Gm^x (SERA DILUTED 1/4 THROUGH 1/128)

Sample	No.	$\bar{X}$	$\sigma_{\bar{X}}$	Range
Gm (x+)	17	4.3	0.4	2–7
Gm (x−)	18	14.6	0.6	11–19
Yoruba	12	9.5	0.3	8–11

ian Aborigines from the Western Desert appear to lack Gm^b entirely (Steinberg, Kirk, and Vos, unpublished).] It is interesting to note that the Gm^b frequency in North American Indians is probably much lower than that in South American Indians (tables 2 and 3) although the South American Indians also have a relatively low frequency of Gm^b; it being 90 per cent or higher in other populations.

The North American and the South American Indians have a higher frequency of Gm^x than other populations thus far reported, and an absence of Gm-like.

The Africans, except the Bashi, are 100 per cent Gm (a+) and Gm (b+), while the Micronesians are 100 per cent Gm (a+) and essentially 100 per cent Gm (b+) (tables 2 and 3). The data for the Bashi, the Pygmies, the Djuka Negroes, and the Javanese are similar (tables 2 and 3) as indeed are the published data for other Negroid populations (see Ropartz, 1960, for review).

African populations, and the Javanese, Micronesians, and the U.S. Negroes show a high frequency of Gm-like, reaching 100 per cent in the Yorubas and the Pygmies. Similarly, they all show a complete absence of Gm^x.

The variability in the frequency of the Gm factors among the populations sampled indicates that they will be valuable adjuncts in the characterization of different human populations. Larger samples and the study of more populations are required before any firm statements concerning the geographic and racial distribution of the Gm factors can be made. However, the present samples permit us to say provisionally, that whites are relatively low in Gm^a, high in Gm^b and Gm^x, and have no Gm-like; the African and Oceanic populations studied are high in Gm^a, Gm^b, and Gm-like, and probably have no Gm^x; North American Indians are high in Gm^a and Gm^x (higher than whites), very low in Gm^b, and have no Gm-like. Eskimos have a similar pattern, but may have less Gm^x and more Gm^b. The small samples of South American Indians indicate that they also are high in Gm^a and Gm^x, lower in Gm^b than non-Indian and non-Eskimo populations, and have no Gm-like.

The data are difficult to interpret in terms of genotypes. Consider first only Gm^a, Gm^b, and Gm^x. In white populations these factors seem to be due to alleles Gm^a, Gm^{ax}, and Gm^b (Harboe, 1959, and Harboe and Lundevall, 1959). Thus the possible genotypes are Gm^aGm^a, Gm^aGm^{ax}, $Gm^{ax}Gm^{ax}$, Gm^aGm^b, $Gm^{ax}Gm^b$, and Gm^bGm^b. Family studies confirm this (Harboe and Lundevall, 1959; Ropartz, Rivat, and Lenoir, 1961, and Steinberg, unpublished). The population data in the present report also support this interpretation.

The maximum likelihood equations for estimating the frequencies of the three alleles, Gm^a, Gm^{ax}, Gm^b, in the white population are as follows (It is assumed that the population is in Hardy-Weinberg equilibrium.):

If we let the frequency of

$$\begin{aligned} Gm^a &= \mathrm{p}, \\ Gm^{ax} &= \mathrm{q}, \\ Gm^b &= \mathrm{r}, \end{aligned}$$

the equations for the maximum likelihood estimates of these frequencies are:

$$\mathrm{p} = 1 - \mathrm{q} - \mathrm{r},$$

$$\mathrm{q} = 1 - \sqrt{1 - \overline{\mathrm{Gm}}(\mathrm{a+\ b-\ x+}) - \overline{\mathrm{Gm}}(\mathrm{a+\ b+\ x+})},$$

$$\mathrm{r} = \frac{\overline{\mathrm{Gm}}(\mathrm{a+\ b+\ x-}) + \overline{\mathrm{Gm}}(\mathrm{a+\ b+\ x+})}{2} + \overline{\mathrm{Gm}}(\mathrm{a-\ b+\ x-}),$$

where $\overline{\mathrm{Gm}}$ refers to the frequency of the indicated phenotype. The estimates based on the data in table 2 are p = .204, q = .092, and r = .704. The expected numbers for each of the five phenotypes listed in table 2 may be computed from these gene frequencies. A comparison of the observed and expected numbers shows that the five phenotypes occur with frequencies in close agreement with the predicted frequencies (table 5).

In Negro populations, which are 100 per cent Gm^a and 100 per cent Gm^b positive and have no Gm^x, it is likely that the Gm^a and Gm^b factors are due to a single allele, Gm^{ab} (Steinberg, Stauffer, and Boyer, 1960). Populations like the Indians and the Eskimos, which are 100 per cent Gm (a+) but show some frequency other than 100 per cent of Gm (b+), require the assumption that they

TABLE 5. COMPARISON OF OBSERVED AND EXPECTED NUMBERS OF THE DIFFERENT Gm PHENOTYPES AMONG U. S. WHITES

(See text for details of calculations)

	+---[1]	+-+-	++--	-+--	+++-	Total
Observed	14	11	87	149	42	303
Expected	12.6	13.9	87.0	150.2	39.3	303.0

$\chi^2_{(3)} = 0.956 \quad .7 > P > .5$

[1] The first symbol (+ or −) refers to Gm^a, the second to Gm^b, the third to Gm^x, and the fourth to Gm-like.

have alleles Gm^a and Gm^{ab}. Since these populations also have Gm^x, and since this factor probably results from an allele Gm^{ax} (Harboe and Lundevall, 1959) it follows that such populations probably have this allele also. Hence, Indian and Eskimo populations probably have alleles Gm^a, Gm^{ax}, and Gm^{ab}.

If we let the frequency of

$$Gm^a = p,$$
$$Gm^{ax} = q,$$
$$Gm^{ab} = r,$$

the maximum likelihood estimates of these frequencies are:

$$p = 1 - q - r,$$

$$q = 1 - \sqrt{1 - \overline{Gm}(a+\ b-\ x+) - \overline{Gm}(a+\ b+\ x+)},$$

and

$$r = 1 - \sqrt{1 - \overline{Gm}(a+\ b+\ x-) - \overline{Gm}(a+\ b+\ x+)},$$

where $\overline{Gm}$ refers to the frequency of the indicated phenotype.

Using the data of table 2 for the 109 Athabascan Indians, the gene frequencies are estimated as $Gm^a = .787$, $Gm^{ax} = .204$, and $Gm^{ab} = .009$. The sample size is too small to test for goodness of fit.

The problem becomes more complicated when Gm-like is included. Thus far we have not obtained families segregating for Gm-like and one of the other Gm-factors, consequently, we do not know its genetic relation to these factors. The observations that the Gm (b+) Gm-like (−) individuals have lower titration scores for Gm-like than do Gm (b−) Gm-like (−) individuals (table 6), and that Gm (b+) Gm-like (+) individuals tend to have a lower titration score for Gm^b than Gm (b+) Gm-like (−) individuals (table 7), suggest that Gm-like may be dependent upon an allele Gm^{bc}, where the 'c' represents Gm-like.

TABLE 6. TITRATION SCORES FOR GM-LIKE

	No.	$\bar{x}$	$\sigma_{\bar{x}}$	Range
1. Gm (b−), Gm-like (−)	5	20.2	0.7	18–22
2. Gm (b+), Gm-like (−)	9	10.7	0.8	8–14
3. Gm (b+), Gm-like (+)	22	2.5	0.4	0–5

Comparison of 1 and 2: $t = 9.4$, $P < 1 \times 10^{-10}$

TABLE 7. TITRATION SCORES FOR Gm^b

	No.	$\bar{X}$	$\sigma_{\bar{X}}$	Range
1. Gm (b−), Gm-like (−)	5	16.8	0.6	15–18
2. Gm (b+), Gm-like (−)	9	7.6	0.7	4–10
3. Gm (b+), Gm-like (+)	22	5.5	0.5	0–11

Comparison of 2 and 3: $t = 2.5$, $P \sim .01$

Populations such as the Yoruba, and the Pygmies which are 100 per cent positive for Gm^a, Gm^b, and Gm-like may have an allele Gm^{abc}.

Only detailed family studies will solve the problems concerning the genetics of the Gm factors.

SUMMARY

Data resulting from the study of the distribution of Gm^a, Gm^b, Gm^x, and Gm-like in U.S. white and Negro populations, in American Eskimo and Indian populations, and in African and Micronesian populations are reported.

Whites have a relatively low frequency of Gm^a, high frequency of Gm^x and Gm^b, and have no Gm-like. The African and Micronesian populations have a high frequency of Gm^a, Gm^b, and Gm-like, and have no Gm^x. North American Indians have a high frequency of Gm^a and Gm^x (higher than whites), a low frequency of Gm^b, and have no Gm-like. Eskimos have a similar pattern, but may have a lower frequency of Gm^x.

The genetic aspects of the Gm factors are discussed. Whites appear to have alleles Gm^a, Gm^{ax}, and Gm^b. It is tentatively suggested that among colored populations, alleles Gm^{ab}, Gm^{bc}, and Gm^{abc} may occur, where c represents Gm-like.

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A Contribution to the Genetics of Hypertrichosis of the Ear Rims[1]

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HOLANDRIC INHERITANCE is defined as a type in which all males are affected and in which females neither exhibit the trait nor transmit the underlying gene. Such type of inheritance is to be expected when a gene with full penetrance of expression is located in and completely linked to the Y-chromosome. Most supposed cases of holandric inheritance in man have been shown either not to fit the definition or at least not to be based on sufficient information to make secure the interpretation in terms of Y-linkage (Stern, 1957).

One of the few human traits, apart from sex, for which complete Y-linkage remains as a possibility is hypertrichosis of the pinna of the ear. Tommasi's pedigree (1907) of an Italian family group fits the interpretation of Y-linkage fully, and only certain doubts about the reliability of the single informant make the pedigree indecisive. A few other pedigrees, of Italian and Indian origin, collected or referred to by Gates (1957) also do not contradict an interpretation in terms of a Y-linked gene but they are too limited to have much weight. Additional data by Gates (1960) on Indian kindreds have not yet been published in detail. Although they are regarded as showing "conclusively that the gene for the hairy ear rims is in the Y chromosome" this statement is subject to restrictions. "In rare cases" there is lack of penetrance and in two cases further assumptions are required such as the "possibility of crossover from Y to X" or other "equally likely" explanations.

Hypertrichosis of the ears is probably more frequent than is generally assumed. Slight degree of the trait can be discerned only by careful observation and stronger expression is often concealed by removal of hairs or shaving. The latter procedure, according to Tommasi, was said to have already been practiced among the younger relatives of the propositus whose hairiness of ears was extreme (Fig. 1).

The present report is based on two groups of data. The first is derived from a study of an Indian population living in villages on the coast of the Bay of Bengal in the subdivision of Contal, District Midnapore, West Bengal. Two of the five pedigrees from this group are from the caste of fishermen (pedigrees 1 and 2) and the remaining three (pedigrees 3–5) from a caste known as Raju who are nearly restricted to the district of Midnapore and to the contiguous dis-

Received November 25, 1960.

[1] Dedicated to Franz Schrader on the occasion of his seventieth birthday.

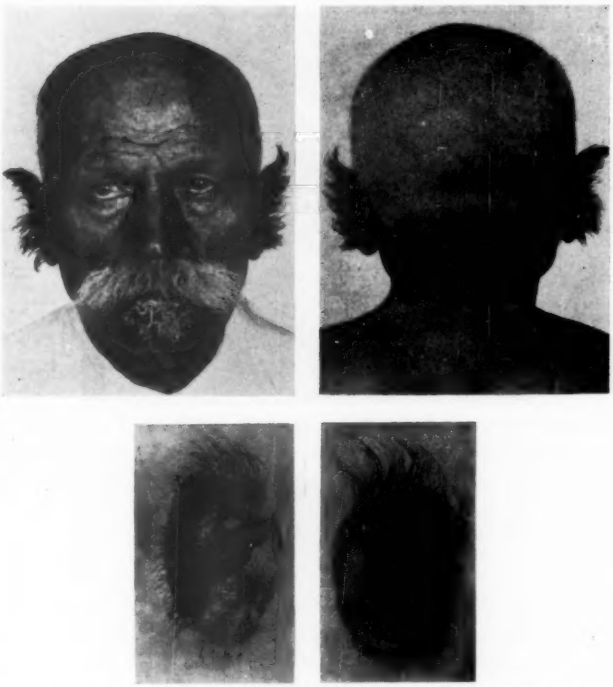


FIG. 1. Hypertrichosis of grade 5 in the propositus of Tommasi's kindred. (Tommasi 1907b).

trict of Balasore in Orissa. The second group of data is represented by the two independent pedigrees 6 and 7 which will be discussed separately.

POPULATION DATA

The variability of the trait among the individuals who possess hypertrichosis of the ears made it advisable to design a rough 5-point scale as follows: 1—very scanty, 2—scanty, 3—medium, 4—marked, and 5—very marked or bushy. The latter, highest degree, is typified by Figure 1 from Italy. No such expression of the traits was encountered in our material. Grade 4 corresponds to that pictured by Gates's (1957) Figures 1 and 2 of the South African boat captain, an Indian originally from Goa. Figure 2, from a Singhalese in Ceylon, provides an illustration of grade 3. Grades 1 and 2 are characterized by the descriptive terms given above (Fig. 3).

A total of 458 individuals in group 1 of the data were examined. None of the 222 adult females or of 10 children of both sexes showed hypertrichosis but 24 out of 226 adult males were affected. The degree of hairiness was of grade 1 and 2 in five of these men, of grade 3 in twelve, and of grade 4 in two. The youngest affected individuals belonged to the age group 20–24 years (table 1).

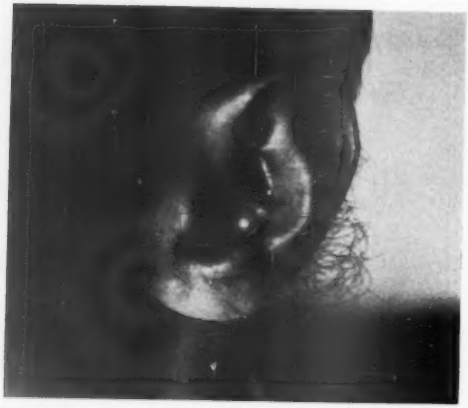


FIG. 2. Hypertrichosis of grade 3 in a Singhalese. Original of Dr. C. S. Coon.

FIG. 3. Hypertrichosis of grade 2 in a Bengalee, age 41, from Calcutta.

The number of cases is insufficient to warrant an analysis of age distribution of the different grades of expression but it is noteworthy that among men of grades 1 to 3 there were some as young as 25–29 years of age or younger, and that one of the two men of grade 4 was in the age class 30–34. All different degrees of expression are thus discernible in early adulthood. The oldest indi-

TABLE 1. DISTRIBUTION OF DEGREES OF HYPERTRICHOSIS IN 24 MALES ACCORDING TO AGE (IN YEARS)

Degree	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–75	75–79	Total
1	1	—	2	—	—	2	—	—	—	—	—	—	5
2	—	2	—	—	1	—	—	1	—	—	1	—	5
3	1	2	—	2	2	2	—	1	1	1	—	—	12
4	—	—	1	—	—	—	—	—	—	—	—	1	2
Total	2	4	3	2	3	4	—	2	1	1	1	1	24

viduals with grade 1 expression were in the 45–49 year group and the oldest ones with grades 2–4 were 60 years of age or older.

No longitudinal studies are available but it is possible that the degree of hairiness might increase somewhat with age.

The 24 affected men constitute about 10 per cent of the male sample. Fourteen of the affected men were not obviously related to one another while the remaining ten were related to one or another of them.

PEDIGREES

Group 1 (Fig. 4)

Detailed information was obtained on five kindreds from group 1 but only a few individuals in each could be seen personally. This leaves many questions unanswered and makes attempts at numerical analysis undesirable. Nevertheless, some important facts can be established. Before commenting on each pedigree it may be stated that illegitimacy is believed to be rare in this population.

Pedigree 1. Only one individual of grade 3 was seen [II-5]. Eight other men were reported as having been affected. The transmission of hypertrichosis in this pedigree is compatible with Y-linkage.

Pedigree 2. Five affected individuals, of grades 3 and 4, and four non-affected males over 20 years of age were seen. It is noteworthy that hypertrichosis entered from two different lines, I-1, I-3, I-5(?), and II-1, II-2. The pattern of inheritance does not conform to simple expectations of Y-linkage.

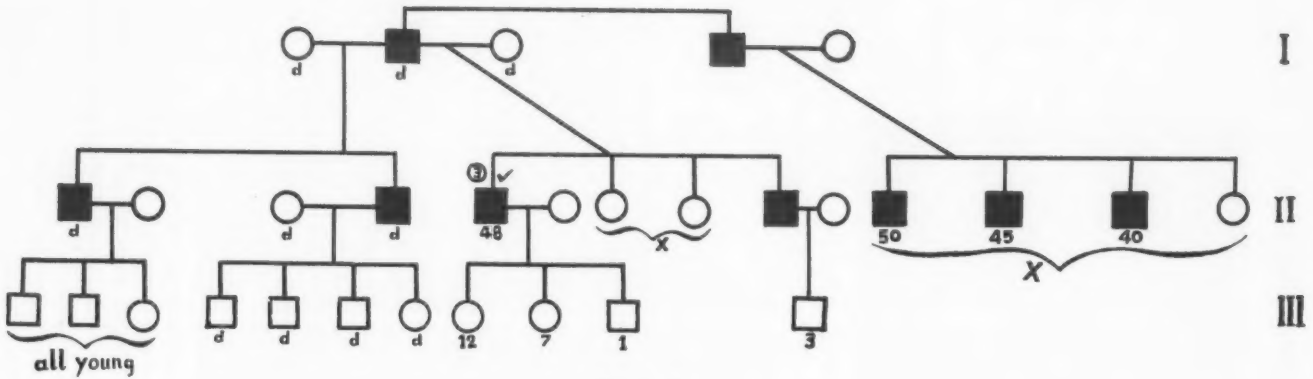
(a) If I-1 was affected, why is his 40 year old son II-3 not affected? If I-1 was actually not affected why was his brother I-3 reputedly affected? If I-3 was actually not affected why were his sons II-5, II-7, and II-9 clearly affected?

(b) Since II-12 was affected, to the degree 3, why were his sons not affected, two of whom were 26 and 30 years old, respectively?

Pedigree 3. Only one affected man, of grade 1, was seen and an older non-affected brother. Y-linkage would have required that the older brother be affected. Additional non-conformance to expectations from Y-linkage is apparent from a number of reputedly non-affected men who are sons of affected fathers, namely II-1, II-2, II-4, II-5 (?), II-9, II-12, and III-2.

Pedigree 4. Only a single person was seen, the weakly affected man II-5, (grade 1). Among his brothers, II-3 and II-6 reputedly were not affected al-

PED. 1.


I
II
III
d
d
d
d
d
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48
50
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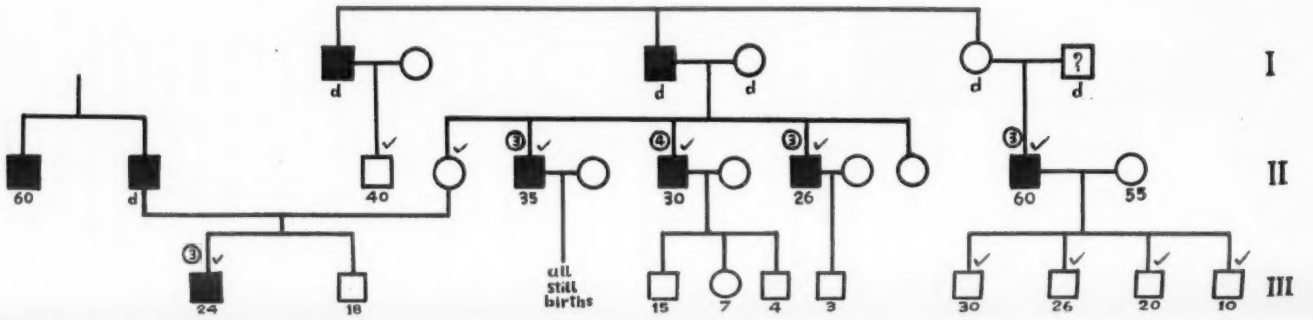
X
X
12
7
1
3
all young

PED. 2.


I
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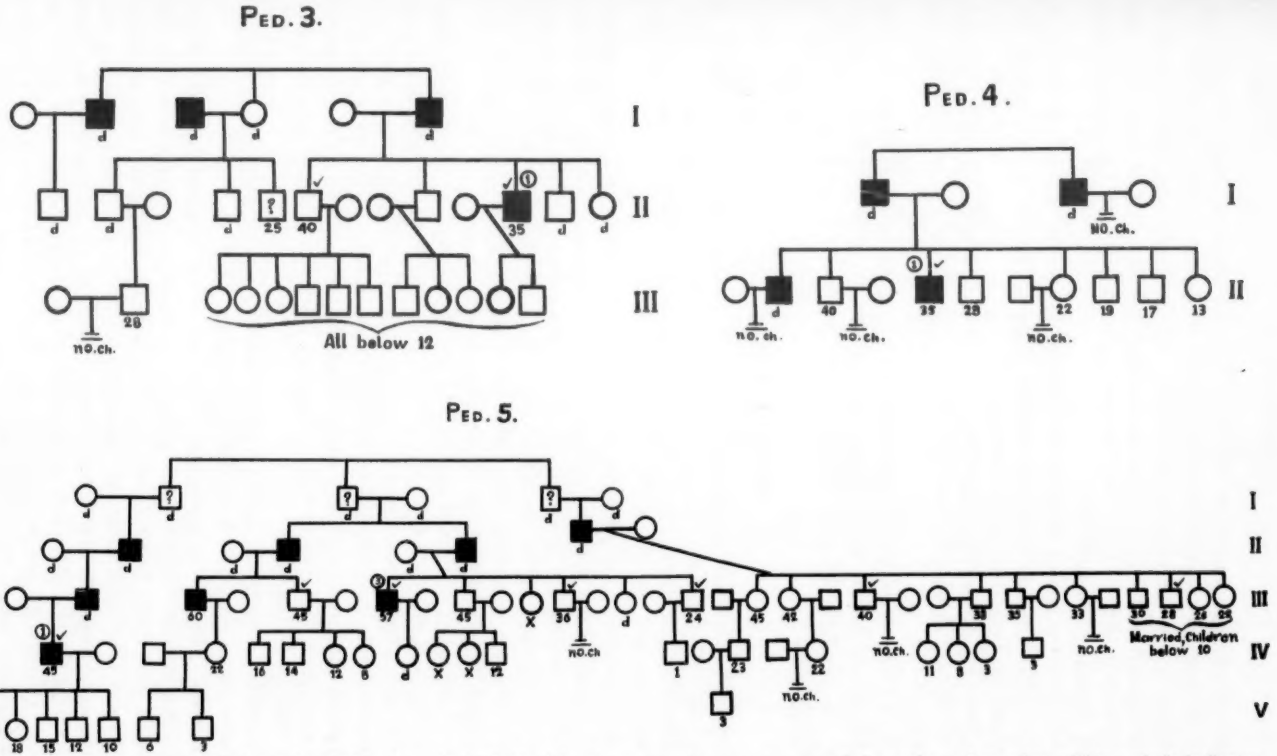

FIG. 4. Pedigrees 1–5. A check mark signifies that the individual has been seen by one or more of the authors. A number within a circle indicates the degree of hypertrichosis. A number below the individual gives the age in years unless specified otherwise. d = dead. x = details unknown.

though one of these was older than II-5 and the other 28 years of age. As it stands this pedigree does not support Y-linkage but the evidence is scanty.

Pedigree 5. In this extensively studied family group two affected men of grades 1 and 3 respectively were personally seen. Five not affected men were also examined. Again, there are various contradictions to expectations from simple Y-linked inheritance.

(a) If II-4 was indeed affected why was his 45 year old second son III-5 not affected? If II-4 was actually not affected why was his son III-3 reputedly affected and II-4's brother II-6 affected?

(b) Since III-7 was clearly affected (grade 3) why were his younger brothers aged 45, 36 and 24 not affected (the oldest reputedly so, the two younger ones certainly)?

(c) If II-7 was affected why were none of his sons, including the two examined men III-20 and III-28, aged 40 and 28, respectively, affected? If II-7 was actually not affected why were his cousins in the male line II-2, II-4, II-6 reputedly affected?

Group 2 (Fig. 5)

Pedigree 6. The kindred represented in this pedigree inhabits a village in Orissa but some of the individuals are now away from their home district. Only one individual, the affected man II-3 (grade 4) was seen. Most of the information was obtained from him.

In this pedigree, as in pedigree 2, hypertrichosis entered from two separate sources, namely I-2 and I-3 on the one side and I-5 on the other. The phenotypes of the three sons and the daughter of I-3 conform to expectation from Y-linkage. No such conformity exists among the children of the sons: Three of these children are now men older than 25 years, namely III-7 aged 35, III-9 aged 26 and III-14 aged 27, but all three are reported to be not affected.

There are three successive generations of affected males in the rightmost part of the pedigree, I-5, II-8, and III-18. This fits Y-linked inheritance but does not contradict other modes of transmission. The wife of II-8 herself has an affected father and affected brothers and could be a carrier of a genotype not expressed in females but expressed in her son, or she could be of a genotype whose segregate jointly with that of a sperm from her husband would lead to hypertrichosis in her son.

In summary, pedigree 6 contains parts which are compatible with Y-linkage and parts which do not fit holandric expectation.

Pedigree 7. This is a pedigree from a village in the District Birbhum, West Bengal. Again, hypertrichosis enters this kindred from two separate sources, the sibship of generation I and III-4. Two affected men were seen, both of grade 3, II-13 and III-4 and numerous not affected individuals. There are two contradictions to simple Y-linked transmission. Neither of the two elder brothers of I-5 nor of the two older still living brothers of II-13 was affected. And it is pertinent that the 28 and 24 year old sons, III-5 and III-7, of II-11, were unaffected as their father, but unlike their affected paternal uncle II-13 and their paternal grandfather I-5.

PEDIGREE 6.

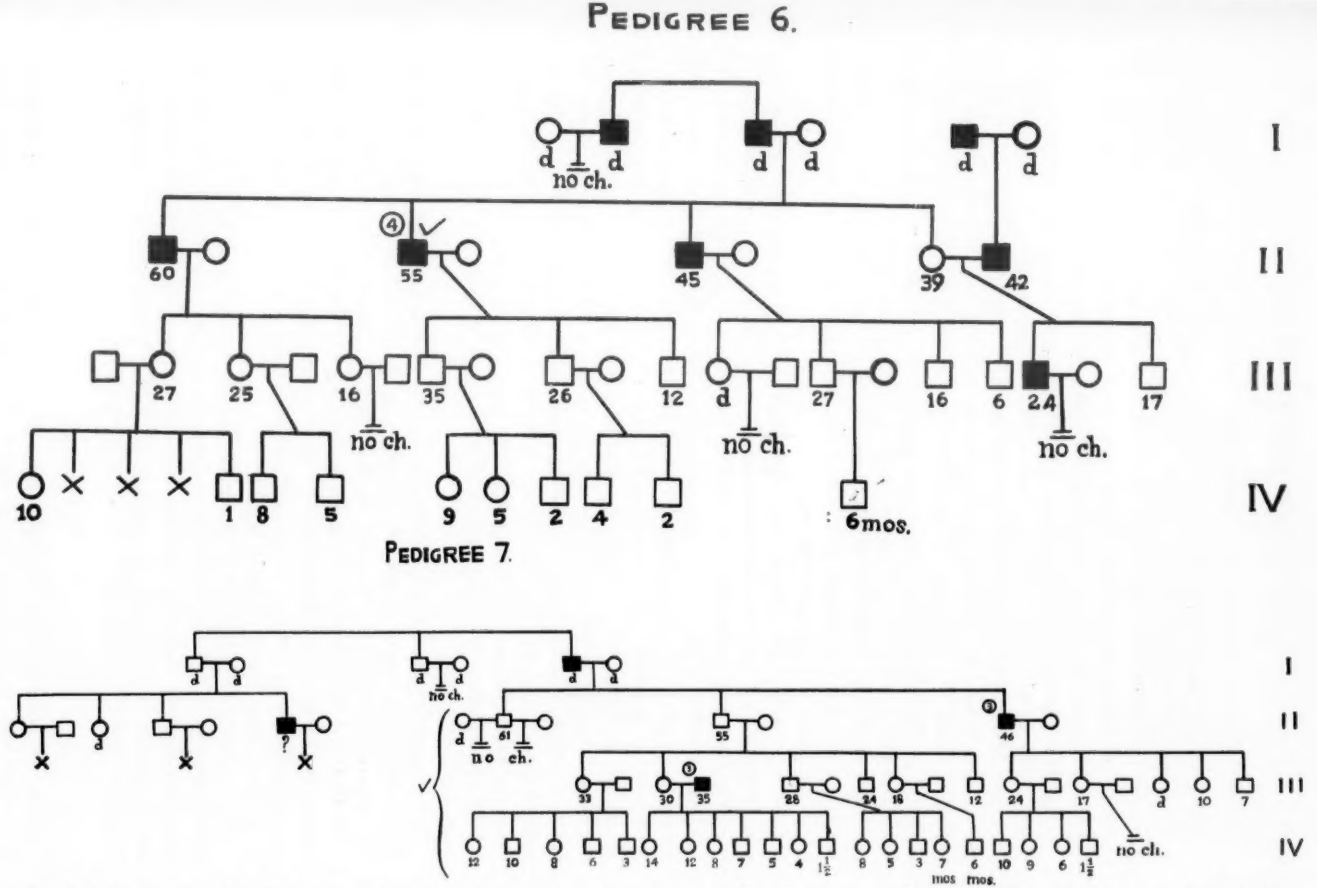

FIG. 5. Pedigrees 6 and 7. For explanation of symbols see legend to Figure 3. The bracket in pedigree 7 signifies that all living individuals to its right have been examined by one of the authors (A.R.B.).

DISCUSSION

The material presented in three of the five pedigrees of group 1 is adequate to eliminate an interpretation of simple, complete Y-linkage of hypertrichosis. A fourth pedigree while most limited in reliable information also does not fit this interpretation. Only one pedigree (no. 1) is compatible with it. Neither of the two pedigrees in group 2 exhibits holandric inheritance.

In these pedigrees various grades of expression of the trait were observed. In two of them two or more affected men were seen. In pedigree 2, two of three brothers belonged to grade 3, and one, the middle, to group 4. Two other men, from two fathers who were not related to those of the three brothers, also belonged to grade 3. In pedigree 5 one of the two affected men belonged to grade 3, the other his cousin once removed in the male line, to grade 1. It is thus clear that the expressivity of a supposed single hypertrichosis gene in a given pedigree may vary. This variation could theoretically include non-penetrance which might account for the absence of the trait in men who, according to the hypothesis of Y-linkage, should carry the gene.

It is regrettable that hardly any information could be obtained about the adult offspring of sisters or daughters of affected men. In the majority of cases the sisters are married outside the villages. In the future it would be desirable to find out whether adult males from non-affected fathers and whose maternal uncles were affected never show the trait as demanded by the hypothesis of Y-linkage or whether they do sometimes. Gates (1960) refers to three sibships in one of his pedigrees in which the daughters of affected men had a total of ten sons (ages 33 to 54) who are unaffected. He regards this as evidence in favor of holandric inheritance. It must be said, however, that the probability of none of three daughters of affected men carrying a possibly autosomal dominant allele for hypertrichosis with male-limited expression is .125 and that the probability of a daughter carrying such an allele but not transmitting it to a few sons is also considerable.

The complete absence of affected females in this population as well as in the kindreds previously described by others, in itself, has no definite bearing on the type of inheritance. Absence of the trait in females is compatible with Y-linkage but equally so with male sex-limited expression of an autosomally transmitted gene. It would be interesting to study sisters of affected men, who on account of overactivity of the adrenal cortex exhibit virilism. Are the ears of some of them hairy as their faces?

The problem of Y-linkage of the genotype for hypertrichosis of the ears remains undecided. If there should be convincing evidence for complete Y-linkage in some pedigrees there are certainly others, as shown in this report, which do not fit readily in this class. In any case it is likely that genetic heterogeneity exists for hypertrichosis as for so many other traits.

SUMMARY

Data are presented on the frequencies of various degrees of hypertrichosis of the ear rims in an Indian population in West Bengal. Five pedigrees from

this population and two pedigrees from different sources are analyzed for evidence of Y-linked transmission of a gene for hypertrichosis. Only one pedigree is compatible with, but does not prove the correctness of, the assumption of complete Y-linkage of a fully penetrant gene. The other six pedigrees do not fit this assumption.

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Blood Group Interaction and the World Distribution of the ABO Gene p^2 and the Rh Gene r (cde)

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THERE HAS BEEN for many years speculation about the role of natural selection in maintaining the frequencies of blood group genes in human populations. Before the discovery of the rhesus blood group system most workers believed that the blood group genes were selectively neutral. In consequence the striking differences which were demonstrated between populations in various parts of the world with respect to the ABO blood group frequencies were considered to be due to the operation of random genetic drift, or to the multiple origins of modern man. The change in attitude to this problem during the last two decades has been summarized by Mourant (1959). Clear-cut evidence for the operation of selection within a blood group system was presented by the demonstration of the role of the Rh blood groups in causing hemolytic disease of the newborn. Early attempts to explain the existing frequency of Rh negative persons in various populations in the face of loss of Rh genes resulting from the birth of heterozygous children to Rh negative mothers dismissed the possibility of adequate replacement by mutation (Fisher *et al.*, 1944). It has been suggested that existing frequencies in European populations are the result of hybridization between stocks of high and low frequency of the gene r (*cde*), and that the present frequency of r is changing under selective pressure (Haldane, 1942: Wiener, 1942). Alternatively, Glass (1950) has presented evidence that at least in persons of European stock where the average family size is small, mothers of children with hemolytic disease compensate by having larger families than mothers not so affected. On theoretical grounds, however, Li (1951) concludes that a compensatory mechanism of this type could not lead to a state of balanced polymorphism.

The renewed interest in selective mechanisms in the Rh blood group system has led to a re-examination of the problem of the ABO blood groups. Careful clinical studies have revealed that incompatibility within the ABO blood group system can result also in hemolytic disease of the newborn (Mollison, 1956; Zuelzer and Kaplan, 1954a; Hsia and Gellis, 1954). The disease is less frequent in European populations than hemolytic disease of the newborn due to Rh incompatibility (Levine *et al.*, 1956) and moreover the prognosis in the majority of cases is not so severe as for the comparable rhesus-induced condition. On the other hand several studies have shown that at least some chronic infertility or repeated abortion is associated with ABO blood group incompatibility or dis-

Received January 30, 1961

turbances in the secretion of ABH substances (Levine, 1943; Matsunaga, 1959; McNeil *et al.*, 1957; Behrman *et al.*, 1960; Wren and Vos, 1961).

Finally, Brues (1954) has pointed out that although there is considerable variation in the ABO gene frequencies in different populations in the world, they occupy a portion only of the theoretically possible range for such frequencies. She argues that this restriction is due to the operation of selective pressures and she has constructed a mathematical model to give selection coefficients which could explain the present world distribution of the ABO blood groups. Livingstone (1960) also has computed the fitness of various ABO phenotypes necessary to account for the observed gene frequencies of the A and B genes in West African populations. He obtained values of the same order of magnitude as those calculated by Brues to account for the world distribution.

The work referred to above has been concerned with selection in the ABO and Rh blood group systems, each considered in isolation from the other. It has become clear in recent years however that the ABO and Rh blood group systems interact. Parents who are incompatibly mated for the ABO blood groups are less likely to have children with severe Rh hemolytic disease of the newborn if they are also incompatible for the Rh blood groups, (See Levine, 1958 for a review of the earlier literature). In consequence, therefore, alteration of gene frequencies in the Rh blood group system must lead inevitably to an alteration in gene frequencies in the ABO blood group system. Cohen and Glass (1959) have drawn attention to the implications of this interaction, and have produced a schematic model to illustrate the magnitude of the effects which might be expected in a European population.

The present communication is an examination of a further aspect of the consequences of such an interaction.

A search of the world literature has been undertaken to discover populations which have been adequately tested for both the A_1A_2BO and Rh blood group systems. The information has been collected where possible from the original papers or from the two surveys of Mourant (1954) and Mourant *et al.*, (1958). Since ABO blood group frequencies can show marked fluctuations in relatively short geographic distances (e.g. Mourant, 1950) only surveys which included tests for both the A_1A_2BO and Rh systems have been included. In the case of the former system samples have been retained where χ^2 for goodness of fit does not exceed 6. In the latter system only surveys in which tests with at least anti-C, anti-D and anti-E were carried out have been included. The frequency of r (*cde*) in such populations is likely therefore to be estimated with accuracy.

The imposition of these restrictions leaves some 67 populations which satisfy all the necessary criteria and the data for which are available to the author. These populations are listed in Table 1, together with the gene frequencies for p^2 and r, respective symbols for the genes controlling the A_2 antigen and the rh phenotype.

The two sets of gene frequencies are plotted in Fig. 1. With two notable exceptions, the Swedish Lapps (Allison *et al.*, 1956) and the Norwegian Lapps (Allison *et al.*, 1952) there is a correlation between the overall world frequencies

TABLE 1. VALUES OF p^3 AND r FOR VARIOUS POPULATIONS

Population	Locality	Frequency of Gene p^3	Frequency of Gene r(cde)	Reference
Pagan tribes	Jos plateau	0.019	0.243	11
Nigerians	S.E. Highlands	0.043	0.238	11
Nigerians	S.W. Nigeria	0.035	0.184	11
Bahutu	Central Africa	0.050	0.194	24
Batutsi	Central Africa	0.061	0.217	24
Luo	Kenya	0.036	0.042	3
Kikuya	Kenya	0.066	0.129	3
Amba Pygmoids	Uganda	0.037	0.133	25
Iraqw	Tanganyika	0.035	0.209	4
Hima	Uganda	0.041	0.278	4
Nilotes	Sudan	0.028	0.197	43
Touaregs	Air	0.024	0.158	37
Bantu	Sth. Africa	0.081	0.154	37 and 39
Bushmen	Kalahari Desert	0.049	0	56
Zabidi Arabs	Aden	0.055	0.199	37
Yemenite Arabs	Yemen	0.062	0.130	37
Parsees	Karachi	0.040	0.270	35
Muslims	"	0.049	0.250	35
Sikhs	Nth. India	0.044	0.299	8
Pakistanis	Nth. West Pakistan	0.030	0.244	37 and 39
Kapol Vania	Bombay	0.007	0.292	55
Bhangi Harijans	"	0.038	0.100	55
Cutchi Lohana	"	0.014	0.200	55
Audichya Brahmans	"	0.043	0.308	55
Leva Patidars	"	0.025	0.292	55
Talavia Dubla	"	0.017	0.137	55
Chenchu	Sth. India	0.006	0.072	47
Basques	Biarritz	0.064	0.493	22
"	Mostly Spain	0.041	0.532	10
"	San Sebastian	0.047	0.481	10
Greeks	Greece	0.067	0.274	16
"	Petromagouca	0.073	0.185	37 and 39
Turks	Mersin	0.081	0.308	1
Dutch	Netherlands	0.085	0.390	21
"	Bunshouten & Spakenburg	0.104	0.501	22
Austrians	Vienna	0.056	0.365	37 and 39
Danes	Copenhagen	0.076	0.388	"
English	London	0.056	0.397	"
Italians	Milan	0.034	0.253	"
"	Ferrara	0.048	0.290	"
"	Sardinia	0.091	0.224	"
Latvians	Latvia	0.025	0.366	41
Lapps	Norway	0.356	0.188	2
"	Sweden	0.323	0.201	5
Walsers	Vals	0.023	0.312	26
"	Safien, Tenna & Versam	0	0.515	26
Ainu	Hokkaido	0	0	48
Chinese	N.Y. City	0	0	54
Chinese	Malaya	0	0	44
Thais	Bangkok	0	0	50

TABLE 1—*Continued*

Population	Locality	Frequency of Gene p^2	Frequency of Gene r(cde)	Reference
Melanesians	New Hebrides	0	0	51
Micronesians	Gilbert Is.	0	0	19
Maoris	New Zealand	0	0	45
Polynesians	Cook Is.	0	0	52
Micronesians	Marshall Is.	0	0	46
Papuans	Schouten Is.	0	0	40
Aborigines	Sth. Australia	0	0	49
Eskimos	Hudson Bay	0.010	0	37
Eskimos	Ungava district	0	0	13
Cree Indians	James Bay	0.007	0.082	13
Sarcee Indians	Alberta	0	0	12
Stoney Indians	"	0	0.097	12
Totonacans	Mexico	0	0	6
Tarascans	Mexico	0	0	6
Huastecans	Mexico	0	0	6
Otomis	Mexico	0	0	6
Chamulas	Mexico	0	0	6

for the genes controlling blood group A_2 and the Rh negative group rh (cde/cde). The distribution of the values of p^2 and r is distorted by the large number of populations in which the value of both p^2 and r is zero. Eighteen such populations have been listed in the present compilation. If these are excluded from the computation, together with the two Lapp samples which lie so far removed from the distribution of all other populations in the world as to merit special consideration, the remaining 47 populations give a correlation coefficient between p^2 and r of $+0.30$ ($0.05 > P > 0.01$). If the distribution of these 47 populations is considered together with the 18 for which p^2 and r are both zero, the relationship between these two gene frequencies would appear to be of much greater significance.

If the world populations are divided into continental groups the correlation between the genes p^2 and r within each continental area with the exception of those areas where both values are zero, is greatly reduced. This may be due in part to sampling errors which become relatively more important when the overall range of values of p^2 and r is restricted, or to the small number of samples available at present for one particular area. In addition one might expect differences in populations subject to various disturbing influences such as a) extreme isolation over long periods of time with consequent inbreeding and genetic drift, b) migration from one part of a continent to another, or c) changes in average size of family which will result in differential change in the selection coefficients for the genes p^2 and r.

The highly aberrant values of the two Lapp samples may be the result of genetic drift in these reproductively isolated populations, or alternatively a selective factor of unique characteristics has resulted in the abnormal increase of the frequency of p^2. Livingstone (1960) has suggested some possible mechanisms for gross disturbance in ABO frequencies.

If it is accepted that for the world population there is a biologically meaningful relationship between the frequencies of p^2 and r it becomes necessary to pos-

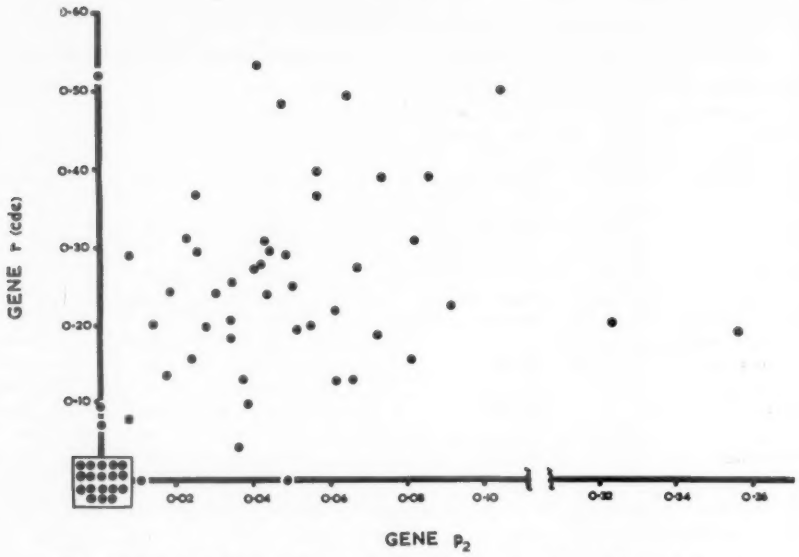

FIG. 1. The distribution of frequencies for the genes p^2 and r in 67 populations.

tulate the nature of the mechanism which controls this relationship. The following scheme suggests tentatively the way in which selection in both the A_1A_2BO and Rh blood group systems could result in the observed correlation.

1. Hemolytic disease of the newborn due to Rh incompatibility negatively selects heterozygous infants to homozygous Rh negative mothers.

2. Sensitization to the Rh antigen is less likely to occur when parents are incompatibly mated for the ABO blood group antigens. Indeed in obligatory incompatible matings, for instance O mothers married to AB husbands or homozygous A or B husbands, Rh sensitization will occur very infrequently unless stimulated by incompatible blood transfusion or extra marital pregnancy.

3. The phenotype A_2 is just as effective as A_1 in reducing the risk of sensitization to Rh antigens.

4. Through the operation of protection against negative selection in the Rh groups, the genes p^1, p^2 and q (p^1 and q being symbols for the A_1 and B antigens respectively) will tend to increase in frequency. This will lead to increased selection due to incompatibility within the ABO blood group system.

5. Within the ABO system A_1 is selected against more severely than A_2.

6. Where Rh incompatibility is tending to select positively for p genes and within the ABO system p^1 is being negatively selected, p^2 will increase proportionately to p^1.

This scheme makes a number of assumptions, not all of which have been validated fully by investigations carried out so far. The following comments seem appropriate however at this stage.

Assumptions numbers 1 and 2 are backed by considerable evidence and need not be discussed further. Assumption number 3 is more difficult to prove since few of the family studies reported in connection with ABO-Rh antagonism have given the sub-types of A. But there seems to be good indirect evidence in its favour. For instance, there is at present no well-authenticated case of a group O Rh-negative mother married to a group AB father becoming sensitized to Rh antigens except where a previous marriage has resulted in compatible children or sensitization has been caused by incompatible blood transfusion (Levine, 1958). Approximately 20 per cent of AB persons should be A_2B. If A_2 does not confer the same protection as A_1 with respect to Rh immunization we should expect to find this proportion of matings of A_2B Rh-positive fathers with group O Rh-negative mothers. Sufficient data has been accumulated in the studies summarized by Levine to make the absence of this particular combination highly significant.

Assumption number 4 raises problems of the magnitude of the effects due to selection in ABO incompatible matings. The papers cited in the earlier part of the present study are relevant to this discussion. It is not even clear whether negative selection operates more severely against A or B before conception (Behrman *et al.*, 1960 and *cf.* Cohen and Glass, 1956) or during pregnancy (Levine 1943, and others) or in the post-natal period due to hemolytic disease of the newborn or other post-natal disorders (*cf.* Struthers, 1951). It is possible that negative selection may be operating in the ABO blood group system at all these stages.

Evidence for assumption number 5 rests solely on the demonstration that A infants suffering from ABO hemolytic disease of the newborn are almost invariably group A_1. Even though they may be typed as A_2 at birth, later retesting shows them to be A_1 (Zuelzer and Kaplan, 1954b).

The deduction in number 6 is consequential on the preceding five assumptions.

It is particularly striking that in those populations in the world, chiefly mongolcid, oceanic and Australian aboriginal, where the frequency of the Rh negative gene r is extremely low or zero the p^2 frequency is also low or zero. At the other extreme, among European populations where the incidence of hemolytic disease of the newborn due to Rh incompatibility is approximately 0.66 per cent of all births the gene p^2 reaches its highest frequency. At this level of Rh incompatibility the effect of ABO-Rh interaction is highly significant. Reepmaker (1955), has calculated on the basis of studies on European populations that there would be an increase of 23 per cent in the incidence of Rh hemolytic disease if the effects of ABO-Rh antagonsism were removed.

Thus it seems clear that in the case of the ABO and Rh blood groups there exists a dynamic equilibrium between the negative selection of two genetically distinct systems and the positive selection resulting from mutual interaction which reflects itself on a world scale in the correlated distribution of the genes p^2 and r. Further detailed analysis of the kind initiated by Cohen and Glass (1959) should lead to a clearer understanding of the role of the p^2 gene in populations where selection against the gene r is still in progress.

SUMMARY

A statistical study of 67 populations in the world for which reliable information on the distribution of the ABO and Rh blood groups is available reveals a significant relationship between the genes controlling the A_2 antigen (p^2) and the Rh negative phenotype (r).

It is suggested that this relationship is a result of the interaction between the ABO and Rh blood group systems which results in positive selection of the gene p^2 in situations where there is negative selection against the Rh gene r.

ACKNOWLEDGEMENT

Financial assistance which made this investigation possible was provided by the Research Grants Committee of the University of Western Australia.

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Population Dynamics of the Sickle-Cell Trait in the Black Caribs of British Honduras, Central America

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INTRODUCTION

THE ORIGINAL purpose of this investigation was to study the ethnic position and the genetic structure of an isolated human population. Such populations provide opportunities for elucidating causal mechanisms concerned in the maintenance of ethnic differences and in the changes in the biological composition of the population known as micro-evolution. The population chosen for study lives along the Atlantic littoral of Central America, from British Honduras south to Nicaragua. These people, known collectively as the Black Caribs, consider themselves as being distinct from any other group found on the American continent. They speak the language of the Island Carib of the Lesser Antilles in the West Indies and have remained socially, culturally, and biologically remote from adjacent non-Carib communities.

The chief emphasis of the present paper is on evidence that the sickle-cell gene has been maintained in this population at frequencies far above those to be expected on the usual assumption concerning the effect of natural selection on a lethal gene. A suggestion is offered on the possible effect malaria in British Honduras may have had in maintaining the high value of the sickle-cell trait observed in the Black Caribs. The observations reported were obtained during four visits (1955–1957) to the Black Carib villages of Stann Creek, Hopkins, Seine Bight, Punta Gorda, and Barranco.

HISTORY OF THE POPULATION

There is a wealth of information concerning the history of the Black Caribs (Edwards, 1806; Young, 1795; Shephard, 1831; Labat, 1742) which is excellently summarized by Taylor (1951). The Black Caribs are said to have originated when slave ships were wrecked, during the 17th Century, on the shores of St. Vincent (Lesser Antilles, West Indies), an island 18 miles long and 12 miles wide. The several hundreds of Africans who survived the wrecks joined the Amerindians who inhabited the island at this time. The Indians, known as the Island Carib, were constantly under attack by Europeans and St. Vincent was one of their last refuges. The "freed" Africans became allies of the Indians in the war against the Europeans. The African population on St. Vincent bor-

Received December 3, 1960.

rowed the dress, customs, culture and language of the Indians and even flattened the heads of their children with frontal and occipital boards. This whole procedure was in the nature of a protective device (Coelho 1955), for these people wanted to be identified as Red Carib Indians, and thus, obviously, useless as slaves. Actually, as Coelho points out, the formation of the Black Carib society as a distinct cultural entity was in reality a response to an historical accident.

The specific provenance of the African progenitors of the Black Carib population is a matter of conjecture. Edwards (1806) believes that a ship wrecked on the island in 1675 supposedly sailed with "Mocoes" from the Bight of Benin, the slaving area of West Africa centering around the mouth of the Niger River. Adams (1801) speaks of the Ibibio Kwas inhabiting a region between the "Bonny" and the Cross River in Nigeria who were called "Mocoes" by white traders.

Whenever the African has come in contact with other ethnic groups in the Americas, there is usually some strong evidence of biological admixture. It was recorded that the Red Carib Indian distrusted their African guests because the latter forcibly abducted Indian women. Labat (1742) mentions that the Red Carib never consented to give their daughters in marriage to these Africans. In time, warfare between the two groups broke out and the Red Carib Indians were reduced, by the early part of the 18th Century, to a few surviving families.

In 1763, the Treaty of Paris gave St. Vincent to the English Crown and in the same year English planters came to occupy the island. A large area of the most fertile lands, however, was reserved for the Black Carib. During the period of the French Revolution, the Black Caribs were profoundly affected by the general upheaval it caused in the West Indies. It was known that they were anxious to adopt the new political system of the French, which, they felt, would guarantee them the right of property. In March 1796, Chatoye, the Black Carib Chief, issued a proclamation announcing adherence of the Black Caribs to the revolutionary program of the French, and they attacked and burned most of the English plantations on St. Vincent.

The Black Caribs, as a nation, were finally forced to surrender to the English in November of 1796, and they were carried off to a small penal island in the Grenadines. In March 1797, a total of 5080 men, women and children were forcibly transported to Roatan, an island off the coast of Spanish Honduras, 2300 miles to the west. The Black Caribs immediately started to migrate to other portions of Central America, some settling in Belize Territory (now British Honduras) as early as 1802. In 1821, the Superintendent of Belize Territory granted the Black Caribs the right to settle in the southern part of the country, which was then uninhabited. Subsequently, immigrants rushed to this new area. Immigration generally ceased by the middle of the 1800's and the growth of the Black Carib population in British Honduras for the past one-hundred years was a result of natural increment (table 1). Estimates of the size of the total Black Carib population, now occupying 400 miles of coast line in Central America, range from 20,000 (Conzemius 1928) to 50,000 (Coelho 1955). They live in some 25 settlements in Nicaragua, Honduras, Guatemala, British Honduras and the island of Roatan.

TABLE 1. POPULATION SIZE OF BLACK CARIBS IN BRITISH HONDURAS

Census Year	Number
1881	2037
1921	3165
1946	4711
1956	6154[1]

[1] Projected population size based on birth and death certificates from 1946–1955.

British Honduras was chosen as the locality for this study. The five Black Carib villages although isolated, were relatively accessible by boat, and there was no difficulty in distinguishing the Black Carib from other Negroid populations. These people were found living in areas set aside as Indian reserves on which they obtained special land grants rights due to them as aboriginal natives. Thus they were politically separated as a group from contiguous communities of "creoles" (the local term for people of African extraction), who also settled in the Colony. Black Carib informants state that only in exceptional cases will a Black Carib marry outside the group or bear children of the "creoles". The individuals and the children of the few mixed marriages that do exist are not considered as belonging to the Black Caribs and generally they live outside the environs of the group.

Three of the five Black Carib communities (Stann Creek, Hopkins, Seine Bight) were studied intensively. Stann Creek may be considered a cosmopolitan Black Carib town, in the sense that the majority of the people, although born there, represent, through their ancestors, almost every Black Carib village. The estimated size of the Black Carib population of Stann Creek and the adjacent village of Hopkins is 4400. The isolated village of Seine Bight contains some 800 inhabitants, all Black Caribs. The majority being descendants of four or five families who settled there from Spanish Honduras in the early 1800's.

BLOOD GROUP GENE FREQUENCIES

A serological study was carried out to determine the frequencies of blood group genes in the population. The blood samples were collected by venipuncture and they were preserved in sterile Alsever's solution in 10 ml vials. The bloods were iced from the time of collection and usually not more than five days elapsed between their collection and their arrival at the laboratories in New York City. Examination of the blood samples were carried out in the serological laboratories at Mt. Sinai Hospital under the supervision of Dr. Richard E. Rosenfield.

The first problem was to estimate, if possible, the degree of admixture between the Island Red Carib and the African, which presumably took place during the middle and late 1600's on the island of St. Vincent. It became apparent that under unusual historical circumstances, the Black Caribs retained the African genotype and virtually all of the Island Carib's social and economic institutions. It is certainly evident from the physical appearance of the Black Carib that the pace of biological hybridization between the two racial groups did not parallel that of the intensive acculturation which occurred. Table 2

gives the gene frequencies of the more important blood group systems tested. The critical feature of the serological results of this study is the identification of the Black Carib population as essentially West African in origin. The presence of relatively high values of R^0 (.404–.437) and r (.306–.337), as well as high B (.134) and A (.131), point to this conclusion although the frequency of r of the Rh blood group system is at the extreme high range for a West African population.

There is evidence of only a small amount of gene flow from Indian ancestors. The blood factor Diego (Di^a) has been identified in nine individuals out of 283 tested with anti-Diego sera, giving a gene frequency of .016. The gene Di^a has been found to be common (.150) in Amerindian mainland Carib populations (Layrisse, 1958) and absent in African populations (Layrisse and Arends, 1957; Gershowitz, 1959). Aside from the Di^a gene we have estimated that R^z of the Rh blood group system, a gene that has not been identified in African populations, has a frequency of .013–.019 in the Black Caribs. The only possible source for both genes was the Island Carib. These data did not permit any precise estimate of the amount of hybridization between the racial groups, but a weighted estimate based on all blood group systems yielded a value of 6–10 per cent admixture between the Island Carib and the West African. Actually what we are measuring is the frequency of survival of introduced genes, not a true rate of gene flow. There is a chance that a certain percentage of the Indian genes would be randomly lost in the ten generations that have intervened since the supposed admixture occurred.

It is not the purpose at this time to compare the gene frequencies of blood groups obtained from the Black Caribs with the values known for specific African populations. Table 2 is presented simply as a means of describing this population through some genetic parameters. Tests of homogeneity between the Black Caribs of Stann Creek and the population living in Seine Bight indicate a significant difference between the two groups for the Rh blood group system (Stann Creek N = 223: Seine Bight N = 212, $\chi_5^2 = 30.2$, $P < .001$); the ABO system (Stann Creek N = 736: Siene Bright N = 150, $\chi_1^2 = 8.8$, $P < .003$) and for S and s of the MNS system (Stann Creek N = 662: Seine Bight N = 151, $\chi_1^2 = 26.2$, $P < .001$). The differences between the two Black Carib populations are not surprising, for they exemplify a principle associated with populations having a restricted "founder" group. The Seine Bight values represent a small original sample, so to speak, from the gene pool of the total population. Most of the other smaller villages, it is suspected, will show the same phenomenon. The gene frequencies indicated for the Stann Creek sample may be considered to be more representative (because of the cosmopolitan nature of the town) of the entire Black Carib population.

FREQUENCIES OF HEMOGLOBIN VARIANTS

During the early phases of this study it was discovered that the Black Caribs of British Honduras had the highest sickle-cell trait frequency yet found in the New World. It is known that the abnormal hemoglobin allele, Hb^S, is main-

TABLE 2. BLOOD GROUPS OF THE BLACK CARIBS

A. Rh Phenotype Frequencies[1]

Phenotype	Stann Creek N = 278 January 1956		Stann Creek N = 223 Summer 1957		Seine Bight N = 212 Summer 1956	
	No.	%	No.	%	No.	%
ccdee	26	9.3	25	11.2	15	7.1
Ccdee	8	2.9	7	3.1	1	.5
ccDee	127	45.7	98	43.9	120	56.6
CcDee	43	15.5	33	14.8	16	7.5
ccDEe	47	16.9	40	17.9	52	24.5
CCDee	2	.7	1	.5	0	0
ccDEE	5	1.8	6	2.7	5	2.4
CcDEe[2]	19	6.8	12	5.4	3	1.4
CCDEe	1	.4	1	.5	0	0

B. Rh Gene Frequencies
(Maximum Likelihood Values)[3]

Allele	Stann Creek (1956)	Stann Creek (1957)	Seine Bight (1956)
R^0 (*cDe*)	.437	.404	.533
r (*cde*)	.306	.337	.267
R^1 (*CDe*)	.074	.075	.039
r' (*Cde*)	.044	.038	.008
R^2 (*cDE*)	.120	.133	.153
R^z (*CDE*)[4]	.019	.013	.000
χ_4^2 [5]	3.68	4.54	.54
P	.5 > P > .4	.4 > P > .3	1.0 > P > 0.9

C. ABO Phenotype Frequencies

Phenotype	Stann Creek N = 282 January 1956		Stann Creek N = 454 Summer 1956		Stann Creek N = 223 Summer 1957		Seine Bight N = 150 Summer 1956	
	No.	%	No.	%	No.	%	No.	%
A_1	43	15.2	59	13.0	40	17.9	39	26.0
A_2	20	7.1	38	8.4				
O	142	50.4	251	55.3	127	57.0	90	60.0
B	69	24.5	94	20.7	48	21.5	18	12.0
A_1B	4	1.4	5	1.1	8	3.6	3	2.0
A_2B	4	1.4	7	1.5				

[1] Tested with five anti-sera: C, c, D, E and e.

[2] Phenotype Class CcDEe when tested with anti-f:

f− = $Rh_1\ rh_2$; $rh'\ Rh_2$
f+ = $Rh_z\ Rh_0$; $Rh_z\ rh$

N = 278: f− = 14: f+ = 5
N = 223: f− = 6: f+ = 6
N = 212: f− = 3: f+ = 0

[3] Maximum likelihood method after Ceppellini *et al.*, (1955).

[4] R^z calculated after Mourant (1954)
R^z counted utilizing anti-f:

N = 278: R^z = .011
N = 223: R^z = .016
N = 212: R^z = 0

[5] χ^2 values refer to the fit of the data to Hardy-Weinberg ratios.

TABLE 2—*Continued*

D. ABO Gene Frequencies
(Maximum Likelihood Values)

Allele	Stann Creek N = 282	Stann Creek N = 454
P_1	.088	.073
P_2	.048	.055
q	.148	.125
r	.716	.747
χ_2^2	1.92	1.68
P	.4 > P > .3	.5 > P > .4

Allele	Stann Creek 1956 Total N = 736	Stann Creek N = 223	Seine Bight N = 150
p	.131	.114	.151
q	.134	.134	.073
r	.735	.752	.776
χ_1^2	1.73	.26	.04
P	.2 > P > .1	.7 > P > .6	.9 > P > .8

E. MNS Phenotype Frequencies

Phenotype	Stann Creek N = 275 January 1956 No.	%	Stann Creek N = 387 Summer 1956 No.	%	Seine Bight N = 151 Summer 1956 No.	%
M S	26	9.4	39	10.1	15	9.9
M s	58	21.1	66	17.0	21	13.9
M N S	35	12.7	63	16.3	46	30.5
M N s	86	31.3	122	31.5	32	21.2
N S	15	5.5	27	7.0	19	12.6
N s	52	18.9	65	16.8	18	11.9
M S^u	2	.7	2	.5	0	0
M N S^u	1	.4	2	.5	0	0
N S^u	0	0	1	.3	0	0

F. *MNS* Gene Frequencies
(Maximum Likelihood Values)

Allele	Stann Creek N = 275	Stann Creek N = 387	Seine Bight N = 151
M S^u	.084	.068	.000
M S	.091	.106	.136
M s	.360	.344	.360
N S^u	.019	.046	.000
N S	.058	.077	.178
N s	.388	.359	.326
χ^2	3.49	.38	2.43
d.f.	3	3	2
P	.4 > P > .3	.95 > P > .90	.3 > P > .2
M	.535	.518	.497
N	.465	.482	.503
S	.149	.183	.314
s	.748	.703	.686
S^u	.103	.114	.000

tained in certain populations at relatively high values despite the fact that this gene is essentially lethal when homozygous (Neel, 1953). This leads to the expectation that some mechanism exists which involves a selective advantage of the heterozygote (Allison, 1954c). The idea of some selective advantage of heterozygotes in the Black Caribs was made more plausible when it was discovered in the preliminary survey of 1956, that the heterozygous mothers had, on the average, more children than the normal homozygous mothers. The further investigation of this population revolved around a program designed to verify this observation.

The blood samples of Black Carib adults submitted for hemoglobin type determinations were routinely run on a paper electrophoretic apparatus in the laboratory of Dr. S. Lee at Mt. Sinai hospital, but the 1957 specimens were also analyzed by the author, in the laboratories of the Institute for the Study of Human Variation at Columbia University, by means of the "starch-block" electrophoresis method (Kunkel *et al.*, 1957). Table 3 lists the results obtained from various collections, and it is to be noted that these samples are consistent in showing a high value of the sickle-cell trait. The summary of this table includes only the normal homozygous ($Hb^A\ Hb^A$) and the sickle-cell trait individuals ($Hb^A\ Hb^S$). Eighteen $Hb^A\ Hb^C$ individuals were also found, but because of their small number they have been omitted from further analysis. The data indicate that adult males have a higher incidence of the sickle-cell trait (.271) than adult females (.218). The Chi Square value for 1 degree of freedom is 2.14 ($P > .05$) which is not significant but is consistent with the evidence to be reported below of a higher incidence in males than females.

THE NUMBER OF CHILDREN BORN TO MOTHERS OF DIFFERENT GENOTYPES

Data on the reproductive performance and sex of offspring of the mothers in the population were secured by interviews. The female population obtained for this study was first selected from mothers who attended the childrens' clinic at the Stann Creek Hospital. Their sisters, whether or not they had children, and their mothers were asked also to appear at the clinic. Older women as well as those not selected through the hospital clinic were obtained by door to door canvassing; a special effort was made to bring in for interview and blood examination women who were in their 70's or 80's. The women examined in the village of Seine Bight represent almost a total sample of the population of females over the age of 30. Only a few of the women of this village were unavailable for study. It is to be noted that the selection of females was biased toward mothers, but the sample contained two per cent of women who did not have any children. According to the 1946 census, four per cent of the women over the age of 44 were childless.

All of the interviews with the mothers were conducted in the presence of a Black Carib leader and therefore it was felt that the mothers tried to give answers that were as accurate as possible and that the responses were relatively correct. It was noted that some of the older women failed to recall some of their children who died early in infancy, or, if they remembered, could not recall the

TABLE 3. ABNORMAL HEMOGLOBINS OF ADULT BLACK CARIBS

Sample	Genotype	Number	Per Cent
Stann Creek	Hb^A Hb^A	101	72.2
N = 140 1956	Hb^A Hb^S	36	25.7
Males	Hb^A Hb^C	3	2.1
Stann Creek	Hb^A Hb^A	109	72.2
N = 151 1956	Hb^A Hb^S	31	20.5
Females	Hb^A Hb^C	10	6.6
	Hb^S Hb^C	1	.7
Seine Bight	Hb^A Hb^A	39	69.6
N = 56 1956	Hb^A Hb^S	16	28.6
Males	Hb^A Hb^C	1	1.8
Seine Bight	Hb^A Hb^A	72	79.1
N = 91 1956	Hb^A Hb^S	18	19.8
Females	Hb^A Hb^C	1	1.1
Stann Creek	Hb^A Hb^A	130	79.8
N = 163 1957	Hb^A Hb^S	33	20.2
Females			
Seine Bight	Hb^A Hb^A	46	71.9
N = 64 1957	Hb^A Hb^S	15	23.4
Females	Hb^A Hb^C	3	4.7
Hopkins	Hb^A Hb^A	44	74.6
N = 59 1957	Hb^A Hb^S	15	25.4
Females			
	Summary[1]		
Males			
N = 192	Hb^A Hb^A	140	72.9
	Hb^A Hb^S	52	27.1
Females			
N = 513	Hb^A Hb^a	401	78.2
	Hb^A Hb^S	112	21.8

[1] Genotype Hb^A Hb^C omitted from the summary.

sex. The responses of a small sample (40) of women between 30 and 40 years of age were selected for checking against the official records of births and deaths available at the Government Registry Office in Belize, and it was indicated that the responses obtained by interview were essentially correct. The few omissions consisted of children who died within a week of birth. There is very close correspondence between the data obtained by interview and the population data recorded by the 1946 census (Census, West Indian, 1949). This material will be reported elsewhere.

Table 4 contains the data on the reproductive performance of mothers with

TABLE 4. REPRODUCTIVE PERFORMANCE OF $Hb^A\ Hb^S$ AND $Hb^A\ Hb^A$ MOTHERS

Age	$Hb^A Hb^S$			$Hb^A\ Hb^A$		
	No.	$\frac{L+D^1}{\bar{X} \pm \sigma_{\bar{X}}}$	$\frac{L^2}{\bar{X} \pm \sigma_{\bar{X}}}$	No.	$\frac{L+D}{\bar{Y} \pm \sigma_{\bar{Y}}}$	$\frac{L}{Y \pm \sigma_{\bar{Y}}}$
All	89	6.17 ± .37	4.62 ± .32	254	4.25 ± .19	3.06 ± .14
≤39	39	5.05 ± .42	3.82 ± .32	109	3.39 ± .24	2.53 ± .18
≥40	50	7.04 ± .55	5.24 ± .49	145	4.89 ± .26	3.39 ± .24

[1] L + D ($\bar{X}$ or $\bar{Y}$) = Mean number of children ever born.

[2] L ($\bar{X}$ or $\bar{Y}$) = Mean number of children living at time of survey or who survived to their sixth year of life.

TABLE 5. TEST FOR SIGNIFICANCE BETWEEN THE REPRODUCTIVE PERFORMANCE OF $Hb^A\ Hb^S$ AND $Hb^A\ Hb^A$ MOTHERS

Age	Difference		Fertility Ratio	
	$\frac{L+D}{\bar{X} - \bar{Y} \pm \sigma_{\bar{X}-\bar{Y}}}$	$\frac{L}{\bar{X} - \bar{Y} \pm \sigma_{\bar{X}-\bar{Y}}}$	$\frac{L+D}{\bar{X}/\bar{Y}}$	$\frac{L}{\bar{X}/\bar{Y}}$
All	1.92 ± .41	1.56 ± .34	1.45	1.51
≤39	1.66 ± .48	1.29 ± .37	1.49	1.51
≥40	2.15 ± .60	1.79 ± .52	1.44	1.52

sickle-cell trait ($Hb^A\ Hb^S$) and of mothers without this trait ($Hb^A\ Hb^A$). The material is partitioned into mothers 40 years of age and over who have probably completed their families, and mothers under the age of 40. The distributions of the ages of the mothers in the two groups is uniform: 56 per cent of the mothers in the $Hb^A\ Hb^S$ group are 40 years of age or over; 57 per cent of those in the $Hb^A\ Hb^A$ group. The younger mothers were selected if they started childbearing before 1946, so the majority of them are between 30 and 40 years of age. Table 4 gives the mean number ($\bar{X}$ for $Hb^A\ Hb^S$ and $\bar{Y}$ for $Hb^A\ Hb^A$) of children ever born (column L + D), and of children living at the time of the survey or who survived to their sixth year of life (column L). These data have been analyzed for differences in reproductive performance between the $Hb^A\ Hb^S$ mothers ($\bar{X}$) and the $Hb^A\ Hb^A$ mothers ($\bar{Y}$). The difference ($\bar{X} - \bar{Y}$) is statistically significant (at the .01 level) for all catagories tested as determined by the t test (table 5).

A fertility ratio, f, was calculated ($f = \bar{X}/\bar{Y}$) for the two classes of mothers. It may be noted that all the groups give values of f between 1.44 and 1.52 (table 5). It was decided to accept, for overall use, the fertility ratio (1.45) of the total ever born (column L + D) for all age classes of mothers, since the mortality of children 0–5 years of age was not significantly different for the two classes of mothers. (A t test for differential mortality yielded a value of .56.)

If the fertility ratio, 1.45, is a measure of the selective intensity of the environment against the normal homozygous mother ($Hb^A\ Hb^A$) and in favor of the heterozygous mother ($Hb^A\ Hb^S$), then an equilibrium value for the percentage of heterozygotes ($Hb^A\ Hb^S$) in the population may be obtained. An iterative method has been devised for obtaining this value (table 6). The procedure yielded an

TABLE 6. EQUILIBRIUM FREQUENCY OF Hb^A Hb^S IN BLACK CARIB POPULATION

fertility ratio (f) = 1.45

FINAL ITERATIVE STEP

Mating types		Frequency of Mating Types	Estimated frequency of heterozygotes from previous iterative process = .26340 Expected Number of Mating Types per 100 Couples	f Ratio
Female	Male			
Hb^A Hb^S	Hb^A Hb^S	$(.26340)^2$	6.938	1.45
Hb^A Hb^S	Hb^A Hb^A	(.26340)(.73660)	19.402	1.45
Hb^A Hb^A	Hb^A Hb^S	(.73660)(.26340)	19.402	1.00
Hb^A Hb^A	Hb^A Hb^A	$(.73660)^2$	54.258	1.00

Mating Types		Relative Number of Children Expected (= No. of Matings × f)	Expected Distribution of Children According to Genotype		
Female	Male		Hb^S Hb^S	Hb^A Hb^S	Hb^A Hb^A
Hb^A Hb^S	Hb^A Hb^S	10.060	2.515	5.030	2.515
Hb^A Hb^S	Hb^A Hb^A	28.133		14.066	14.067
Hb^A Hb^A	Hb^A Hb^S	19.402		9.701	9.701
Hb^A Hb^A	Hb^A Hb^A	54.258			54.258
Total:		111.853	2.515	28.797	80 541

$$\text{Frequency of heterozygotes } (X_n) = \frac{\text{number of } Hb^A\ Hb^S \text{ children}}{\text{Total number of surviving children}}$$

$$X_n = \frac{28.797}{109.338} = .26338$$

equilibrium frequency of 26.3 per cent heterozygotes to be expected in the Black Carib population.

Thus the greater number of livebirths of Hb^A Hb^S mothers as compared to Hb^A Hb^A mothers is sufficient to maintain the observed frequency of the sickle-cell trait (.241, table 9). There is no direct evidence of the cause of the differences in numbers of livebirths, but the most plausible explanation is an excess of abortions among the Hb^A Hb^A mothers which may be associated with the presence of malaria. This will be discussed further below.

As was mentioned previously, there is a slight, although not significant, suggestion that the frequencies of the heterozygotes may differ in the two sexes. The data obtained on the reproductive performance of mothers may be used to test whether the sex ratio among the offspring of the two groups of mothers differed. It is to be remembered that information from older women was unreliable regarding the sex of a child who had died early in life. Therefore the following data (table 7) refer to children who reached the sixth year of life before dying or who were alive at the time of the survey. (A special effort was made to secure information as to sex of child from the least numerous class i.e. Hb^A Hb^S mothers. This accounts for the relative increase of the Hb^A Hb^S mothers included in table 7 as compared to table 4.) The fact that there was no significant difference in mortality of children between the two classes of mothers suggests that one should not ex-

TABLE 7. SEX RATIO AMONG OFFSPRING OF Hb^A Hb^S, Hb^A Hb^A MOTHERS AND AMONG OFFSPRING FROM A RANDOM SAMPLE OF MOTHERS

Mothers	No. of Mothers	No. of Children	Males		Sex Ratio
			No.	%	
Hb^A Hb^S	68	331	172	51.96	108.2:100.0
Hb^A Hb^A	95	409	170	41.56	71.1:100.0
Random	171	994	430	43.26	76.4:100.0

pect a great difference between the two groups in the sex of children dying during the years 0–5. Table 7 indicates that offspring of Hb^A Hb^S mothers have a relatively normal sex ratio, 108:100, or 52 per cent males. The offspring of Hb^A Hb^A mothers, on the other hand, have a very low sex ratio, 71:100, or 42 per cent males. These data can be compared directly with the sex of children obtained by a random survey of 171 mothers of unknown genotype: 76:100, or 43 per cent males. The random survey was constructed of families consisting of 4 or more children. It would be more appropriate to compare the sex ratio with the values obtained from the 1946 census (Census, 1949). Table 10 indicates that the population sex ratio is 79:100, or 44 per cent males.

The difference in the sex ratio of offspring of Hb^A Hb^S and Hb^A Hb^A mothers is significant ($\chi_1^2 = 7.96$, $P < .01$). Since the difference in the sex ratios of the offspring is statistically significant we have to consider the effect of this differential upon the equilibrium value of the heterozygote.

Since it has been shown that the sex composition of Black Carib families is different depending upon the presence or absence of an abnormal hemoglobin gene in the mother, it can be expected that the frequency of the abnormal hemoglobin gene will differ in the two sexes. If, as observed, the Hb^A Hb^A and Hb^A Hb^S mothers have a fertility ratio of 1:1.45, and the male sex rate for each group is known, it is possible to calculate not only the equilibrium frequency value to be expected for the sickle-cell trait within each sex, but also the expected distribution of the sexes within the population. An iterative process has been used (table 8) to obtain these equilibrium values. The population equilibrium frequency for the sickle-cell trait becomes .282 in males and .247 in females compared to observed values of .271 in males and .218 in females. Table 8 also gives a theoretical sex ratio at equilibrium of 81:100 derived from the original sex ratios observed for Hb^A Hb^S and Hb^A Hb^A mothers. This sex ratio gives a weighted mean equilibrium frequency of .263 Hb^A Hb^S for the population. Using the observed 1946 census sex ratio (78.7:100) the weighted mean of the observed Hb^A Hb^S frequencies is .241. It should be noted that the osberved frequencies for Hb^A Hb^S males and females are very close to the calculated equilibrium values and that the calculated equilibrium sex ratio is also very close to the census value (79:100).

Previous workers (Allison, 1954–5; Lehman and Raper, 1956) have suggested that the equilibrium frequency of the sickle-cell trait is maintained by differential mortality of infants and children. If the fitness (relative survival) of normal homozygotes is arbitrarily taken as unity, it is customary to denote the fitness of the heterozygote by W. If the equilibrium frequency of the sickle-cell trait in the adult breeding population is q and the frequency of Hb^A Hb^A is $1 - q$, with

TABLE 8. EQUILIBRIUM FREQUENCY OF MALE/FEMALE $Hb^A\ Hb^S$ IN BLACK CARIB POPULATION

FINAL ITERATIVE STEP

Estimated frequency of male/female heterozygotes from previous iterative process =
Males: = .282353
Females: = .246884

Mating Types		Frequency of Mating Types	Expected Number of Mating Types per 100 Couples	f Ratio
Female	Male			
$Hb^A\ Hb^S$	$Hb^A\ Hb^S$	(.246884)(.282353)	6.9708	1.45
$Hb^A\ Hb^S$	$Hb^A\ Hb^A$	(.246884)(.717647)	17.7176	1.45
$Hb^A\ Hb^A$	$Hb^A\ Hb^S$	(.753116)(.282353)	21.2645	1.00
$Hb^A\ Hb^A$	$Hb^A\ Hb^A$	(.753116)(.717647)	54.0471	1.00

Mating Types		Relative Number of Children Expected (= No. of Matings × f)	Expected Distribution of Children according to Observed Sex Distrib. in Hemoglobin Sample of Mothers	
Female	Male		Female	Male
$Hb^A\ Hb^S$	$Hb^A\ Hb^S$	10.1077	5.2524	4.8553
$Hb^A\ Hb^S$	$Hb^A\ Hb^A$	25.6905	13.3498	12.3407
$Hb^A\ Hb^A$	$Hb^A\ Hb^S$	21.2645	8.8386	12.4259
$Hb^A\ Hb^A$	$Hb^A\ Hb^A$	54.0471	22.4647	31.5824
Total:		111.1098	49.9055	61.2043

Mating Types		Expected Distribution of Children according to Sex and Genotype					
		Male			Female		
Female	Male	$Hb^S\ Hb^S$	$Hb^A\ Hb^S$	$Hb^A\ Hb^A$	$Hb^S\ Hb^S$	$Hb^A\ Hb^S$	$Hb^A\ Hb^A$
$Hb^A\ Hb^S$	$Hb^A\ Hb^S$	1.3131	2.6262	1.3131	1.2138	2.4277	1.2138
$Hb^A\ Hb^S$	$Hb^A\ Hb^A$		6.6749	6.6749		6.1703	6.1704
$Hb^A\ Hb^A$	$Hb^A\ Hb^S$		4.4193	4.4193		6.2129	6.2130
$Hb^A\ Hb^A$	$Hb^A\ Hb^S$			22.4647			31.5824
Total:		1.3131	13.7204	34.8720	1.2138	14.8109	45.1796

Expected Distribution of $Hb^A\ Hb^S$

	Frequency
Population average $\frac{28.5313}{108.5829}$	= .262761
male $\frac{13.7204}{48.5924}$	= .282357
female $\frac{14.8109}{59.9905}$	= .246887

Expected Per Cent Distribution of Sex
male: .44751
female: .55249
sex ratio: 81.00:100

TABLE 9. SUMMARY OF ABNORMAL HEMOGLOBIN FREQUENCIES

	Per Cent of Hb^A Hb^S Heterozygotes	
	Observed	Expected
male:	.271	.282
female:	.218	.247
Population average:	.241[1]	.263

[1] Adjusted value with sexes distributed as in the 1946 census (table 10).

TABLE 10. SEX RATIO IN THE BLACK CARIB POPULATION[1]

(males per 100 females)

Age Group	Sex Ratio
0–4	83.17
5–9	81.71
10–14	90.00
15–19	79.96
20–24	76.47
25–29	70.59
30–34	75.62
35–39	73.33
40–44	78.52
45–49	66.67
50–54	70.00
55–59	80.00
60–64	87.67
65–69	70.31
70+	69.70
All	78.72

[1] From 1946 Census.

Hb^S Hb^S effectively absent, it can be shown that W must equal $(2 - q)/(2 - 2q)$. Taking the observed frequency of the sickle-cell trait q = .241 (table 9), we find W = 1.16. Then starting with q = .241, at birth there would be .785 Hb^A Hb^A to .215 Hb^A Hb^S, not counting the Hb^S Hb^S homozygotes who will not enter the breeding population. It can be verified that natural selection with W = 1.16 will then restore the original frequency of .241 for sickle-cell trait in adults. The hypothesis of such differential mortality could then be tested by comparing the frequency of Hb^A Hb^S individuals in adults with that at birth. Unfortunately, very large samples would be necessary. To have a 90 per cent chance that the observed difference would be significant at the 5 per cent level, when the true frequency was .215 in infants and .241 in adults, we would need about 4000 infants and 4000 adults, or many more than exist in the Black Caribs or most of the studied African populations.

On the other hand, the present study has shown a significant difference in fertility of homozygous and heterozygous females. This difference happens to be of just the right order of magnitude to account completely for the observed frequency of sickle-cell heterozygotes. However both the fertility ratio (f) and the

TABLE 11. DISTRIBUTION OF MALARIAL DEATHS IN BLACK CARIBS[1]
(Stann Creek District)

Year	Malarial Deaths 0–5 years		Deaths from All Causes 0–5 years	Malarial Deaths >20 years	
	Male	Female		Male	Female
1932–1936	16	15	120	13	18
1937–1940	17	10	99	17	14
1941–1945	14	4	96	8	5
Total: 1932–1945	47	29	315	38	37

[1] From Death Certificates, Government Registry Office, Belize.

frequency of heterozygotes (q) are subject to large sampling errors, and it is quite possible that part of the selection may involve differential child mortality. Nevertheless the major portion of the selection in the Black Caribs would seen to depend upon differential fertility.

THE ROLE OF MALARIA

It has been suggested that malaria plays a role in governing the level of the Hb^S gene in populations under certain conditions (Allison, 1954a). Now it is necessary to define the malarial situation as it may have existed in British Honduras and also to explore the possibility that malaria may affect in some manner the fertility of Hb^A Hb^A mothers, while Hb^A Hb^S mothers are relatively protected from this effect.

Malaria was an important cause of mortality in British Honduras. Malarial deaths in 1905 were estimated at 811.7 per 100,000 inhabitants. In 1939, the mortality rate was 162.3 and in 1948, 85.6 per 100,000 (British Honduras Medical Report, 1954). In 1950 a program for the eradication of the vectors (*Anopheles albimanus* and *A. darlingi*) of malaria began, and the rate of malarial deaths fell to 9.5 per 100,000 by 1952. These figures are based on the total population of the country, but it should be expected that the actual rate of malarial deaths among the Black Caribs far exceeds these figures, for malaria in the southern part of British Honduras caused more deaths than in the more populous northern section. Table 11 summarizes the reported cases of death from malaria (from death certificates at the Belize Registry Office) among the Black Caribs for the years 1932–1945. In young children (0–5 years of age) malaria caused 24 per cent of the total mortality. (The infant and child mortality represent 17.6–18.9 per cent of the children born during this period.) Of particular interest is the fact that adult mortality due to malaria is also relatively high.

Anopheles darlingi has been identified as the most important vector of falciparum malaria in the New World. This species has a widespread distribution in South America, but there is a small focal population restricted to southern British Honduras (Kemp, 1940; Kumm and Ram, 1941). The association of the Black Carib population with this species assumes importance when it is realized that the highest rates of endemic malaria are due to *A. darlingi* and

were it not for this species, falciparum malaria in the Western Hemisphere would be a disease of relatively minor importance (Gabaldon 1949).

A. darlingi is strongly anthropophilous and may be compared to *A. gambiae* of Africa as a vector. Giglioli (1948) points out that in British Guiana, South America, malaria is man-made due to the agricultural bonification of the land. *A Darlingi*, a natural native of the sheltered forests of the interior, is now established near human habitation. This is, as he explains, "an unhappy marriage of agricultural development and malaria". Livingstone (1958) theorizes that the spread of malaria in Africa occurred through a similar process. He believes that *A. gambiae* is adapted to the environment as modified by man. Thus, only when man opened up the African forests, did falciparum malaria reach endemic proportions. It is apparent then, that in certain parts of the New World and Africa a special complex relationship between parasite, host, and vector, exists. The vector controls, in part, the level of malarial infection in human populations. *A. gambiae* and *A. darlingi* are considered to be vectors of stable or moderately stable malaria. A community exposed to attack by these species builds up a stable immunity where all but the youngest children have experience with malaria, and eventually all are resistant to it.

Since adult Black Caribs died from malarial attacks it is assumed that malaria among the Black Caribs was not stable. Also *A. darlingi* might be a less effective transmitter of malaria at the extreme periphery of its range.

The association of high frequencies of the sickle-cell trait and falciparum malaria in Africa is strongly marked for areas where "hyperendemic" malaria is found (Allison, 1956–7; Neel, 1957). Hyperendemic malaria (Christophers, 1924), is said to occur when there is a perfect balance between infection and immunity in a population, almost amounting to commensalism. Wilson and Garnham (1950) point out that, unfortunately, the term hyperendemic has been applied to conditions in Africa in which the balance is less effective. Here the community has incomplete immunity, possibly because of the lowered transmission rate by the vector (MacDonald, 1957). Thus two entirely dissimilar types of community malaria have come to be included in one classification. It is unfortunate that the "community" basis of immunity to malaria has not been followed by the investigators of the sickle-cell trait in Africa.

The "malaria hypothesis" is predicated on the fact that infant malarial mortality is so extensive in areas of hyperendemic malaria that selection through death of $Hb^A\ Hb^A$ individuals is sufficient, in a population sense, to offset the loss of the Hb^S gene through the death of homozygotes ($Hb^S\ Hb^S$). There is some reluctance on the part of malariologists to believe that malarial mortality is powerful enough to account for the maintenance of the high sickle-cell trait frequencies of 40 per cent observed in some African populations (Lehmann and Raper, 1956). The influence of malaria on infant mortality is especially debatable in hyperendemic (*sensu strictu*) regions. Hackett (1941) and Walton (1947) suggest that infants in a population exposed to this type of malaria might be provided with immune anti-bodies from their mothers and, as Covell (1950) has shown, be protected during the first few months of life by this

passive transplacental immunity. Jelliffe (1952) suggests that this passive immunity allows the infant to build up active immunity to malaria. The dangerous period for a child under these conditions is between the age of six months, when he loses passive immunity, and two years when he has developed a degree of active immunity.

Wilson, Garnham and Swellengrebel (1950) believe that under certain conditions the primary malarial attacks of infants and young children cause little mortality. Macdonald (1951) and Shortt (1951), on the other hand, maintain that under no circumstances is immunity acquired by a population living under hyperendemic conditions except by considerable infant and child morbidity and mortality. Data supporting the idea that malaria is not an important cause of death is supplied by many field observations (Garnham (1949) in Kenya; Swellengrebel (1950) in Surinam, South America; Davis (1948) in Uganda and Patel and Vandergoten (1940) in the Belgian Congo). Conversely, the field data of other competent workers indicate that malaria is an important cause of death in children (Whitbourne (1930) and Smith (1943) in Nigeria; Colbourne and Eddington (1954) in Ghana and Duren (1951) in the Belgian Congo). The inconsistency of the data stems perhaps from the false basis for the classification of hyperendemic areas of malaria, therefore these results are, in a sense, not comparable. Garnham (1949) points to another factor that may also play an important role in controlling the level of infant mortality. He found that in the Luo tribe of Kenya, which inhabits an area that is truly hyperendemic, the infants were all parasitized by the age of 12 months, but the symptoms were characteristically mild. The children appeared to be relatively unaffected by the disease and the mortality rate was negligible. He suggests that, compared to other hyperendemic regions, this population was acquiring an immunity to relatively few antigenically different strains of *Plasmodium falciparum* and so more easily acquired immunity. Thus the disease appeared to be milder than in other areas. Perhaps this is an indication that some African populations that have been investigated for the sickle-cell trait, exist in an environment drastically changed, in respect to malarial community infection, from conditions that might have prevailed in preceding generations. With the increase of malarial strains available for infecting populations, the task is, from a community standpoint, to build up an immunity of a polyvalent nature for malaria (Mackey 1953), and this seems to be more difficult than for populations exposed to only a few antigenic strains of *P. falciparum*. Increased child mortality may be one possible effect of this new situation.

Not only do we have conflicting data concerning the intensity of malaria as measured by its effect on mortality, but reports from Africa are also contradictory concerning the basic tenets of the "malarial hypothesis" in respect to the sickle-cell trait. Support for the "malarial hypothesis" is based on attempts to prove that mortality, presumably from malaria, acts selectively: it assumes that more children die from malaria if they are of the genotype $Hb^A\ Hb^A$ than if they are $Hb^A\ Hb^S$. Heterozygosity ($Hb^A\ Hb^S$) is assumed to confer protection against death by malaria. The majority of the studies have approached

this problem through indirect means (Neel 1956–7), by assuming that if there is a predilection for the Hb^A Hb^A child to be heavily infected, while malarial infestation in the Hb^A Hb^S individual is comparatively lighter, the normal homozygotes should have a higher death rate. This is based on an observation by Field (1949) who has demonstrated that malarial mortality is directly related to the level of the parasite count. There is as yet no direct statistical proof from African data, at an acceptable level of significance for selection in favor of heterozygous individuals, or that malaria is in any manner responsible for the maintenance of high frequencies of the sickle-cell trait in many populations. The inferential evidence however is fairly strong.

It has been indicated above that it is the differential livebirth rate, possibly due to a differential spontaneous abortion rate, that is responsible for the maintenance of the Hb^S gene at equilibrium values in the Black Caribs. It is the purpose of the following section to discuss a possible effect of the Hb^S gene which could account for this.

Allison (1956) showed that red blood cells of Hb^A Hb^S individuals sickle only when the partial pressure of oxygen falls below 15 mm. Hg. This occurs only under unusual conditions. Miller, Neel, and Livingstone (1956) suggest that red blood cells carrying some of the developing forms of *P. falciparum* adhere to the walls of blood vessels or are present only in internal organs. This situation may lower the partial pressure of oxygen within the red blood cell so as to induce sickling in an Hb^A Hb^S individual, thus limiting the extent of malarial infection by the destruction of the red blood cell and the parasite which it contains. Kitchen (1949) states that schizonts, which circulate in the internal organs, are the only plasmodial forms as yet recognized to be actively concerned with pathogenesis in malaria, and it is from the direct effects of their growth that symptoms of malaria originate. Livingstone (1957) suggests that falciparum schizonts might fail to develop in the placenta of heterozygous (Hb^A Hb^S) mothers, while this organ would be a prime source of infection in normal homozygous mothers (Hb^A Hb^A). Clark and Tomlinson (1949) state that because of its large vascular spaces and the slow circulation rate, the placenta after the third month of pregnancy would be an important site for the maturation of parasites and for the infection of other erythrocytes. It could thus be a prime site for the occurrence of the sickling of the red blood cells because of the lack of oxygen. Blacklock and Gordon (1925a; 1925b) found the blood from the placenta loaded with parasites at a level rarely approached in peripheral blood. They also suggest that even in females who have built up an immunity against malaria, the placenta offers a suitable place for the development of the asexual forms of the parasite. These authors conclude that there is some evidence which suggests that malarial infection of the placenta predisposes the mother to accidents during pregnancy. Bruce-Chwatt (1952) found that there was a difference in mean weight of infants from mothers with and without infected placenta. He believes that malarial infection may produce a considerable amount of pathological change in the placenta and that this has a deleterious effect on the child both in-utero and during the first week of life.

There is some support for the idea that it is the type of malaria found in the community that may control the factors inducing abortion. Garnham (1938; 1949) suggests that malaria plays only a small part in influencing abortion among immune groups of mothers while it is an important causative factor for abortion among the non-immune group. Thus the incidence of abortions may be found to be in inverse proportion to the degree of immunity possessed by the mother. Wilson and Garnham (1950) point out that in populations where the immune do not suffer malarial attacks after infancy there is no apparent effect on fertility. However, when the frequency of infection and therefore endemicity falls to such an extent that there are regular attacks of malaria throughout life, there has been observed a reduction in fertility.

The data on the differential fertility between $Hb^A\ Hb^A$ and $Hb^A\ Hb^S$ mothers in Africa have generally proven to be negative; either the body of data reviewed was too small to be significant or the fertility differential, when present, was too low to account for the maintenance of the sickle-cell trait frequencies at the levels observed in the populations studied. Delbrouck (1958) suggests that only in women above 30 years of age does one get significant differences in fertility. However his data reveal that the fertility ratio of the total live births between $Hb^A\ Hb^A$ and $Hb^A\ Hb^S$ mothers is only 1.09 which is not sufficient to account for the sickle-cell trait frequency of .24 in the population he studied. Hiernaux (1952) indicates that the offspring borne of normal by trait unions have a slight but not statistically significant lower mortality than offspring of normal by normal matings. The fertility ratio was 1.09 in this study.

Edington's (1955) data show that mothers with sickle-cell trait have a greater fertility rate and a slightly lower stillbirth rate than normal homozygous mothers and that the offspring of mothers with sickle-cell trait have a higher mean birth weight and a higher survival rate than the offspring of normal homozygous mothers. The fertility ratio for the total live-born in this material is 1.24, which is slightly below the value needed (1.29) to account for a sickle-cell trait frequency of .20. Livingstone (1957) pointed out that if one considers the survivorship data alone from this series, the fertility ratio is 1.34, which exceeds the equilibrum value. However Edington's series is not large and these values are not at an acceptable level of statistical significance. Allison (1956–7) tested his material for a fertility differential between trait by normal, and normal by normal matings and found no differences. Even when an adjustment is made to extract the fertility ratio of $Hb^A\ Hb^A$ and $Hb^A\ Hb^S$ mothers the a ratio is hardly enough (1.10) to explain the sickle-cell trait frequency of .38 found in the population he studied.

To summarize, it may be said that under certain conditions, depending upon the immunity level of the community involved, malaria may be responsible for an increased number of abortions. Giglioli (1948) for example, claims that malaria in British Guiana is the main factor in retarding the natural increment of a population due to a decrease in the number of births. The data from Africa do not seem to suggest that differential fertility alone can be responsible for the elevation of sickle-cell trait frequencies in populations living under

states of intense malaria infestation. Support for the "malaria hypothesis" in Africa is contradictory and most of the studies have been of an indirect nature. However it is to be noted that the specific description of the type of community immunity is lacking from most of these reports. There seems to be an incongruity in the concept that the presence of hyperendemic malaria in certain parts of Africa is correlated with high sickle-cell trait frequencies since the basis of this hypothesis is that selective mortality among children is responsible. Malariologists have questioned the relevance of this deduction since it is believed that infant mortality is negligible in many of the populations that have adjusted to intense malarial conditions by immune reactions.

SUMMARY

The Black Caribs of British Honduras are an essentially biologically identifiable African population transplanted to the New World. In this population a high frequency (.241) of the sickle-cell trait (Hb^A Hb^S) was observed. It has been demonstrated that the sickle-cell gene Hb^S is maintained in this population at equilibrium values due to the greater fertility of the heterozygous (Hb^A Hb^S) mothers (fertility ratio 1.45). The most likely explanation is that normal homozygous mothers (Hb^A Hb^A) have more pregnancy interruptions. The sex ratio of the offspring of Hb^A Hb^A mothers is low, while that of Hb^A Hb^S mothers is normal. It is suggested that because the Black Caribs are exposed to falciparum malaria of medium endemicity, the placenta of infected non-immune mothers might be predisposed to some mechanical injury causing intrauterine death of the fetus. The mothers who are heterozygous for the sickle-cell gene seem to be relatively protected from this type of interaction.

ACKNOWLEDGEMENTS

This study was supported by the Sigma Xi Fund, the Wenner-Gren Foundation for Anthropological Research and the Population Council Inc. The author wishes to thank Drs. R. E. Rosenfield and S. Lee of Mt. Sinai Hospital, New York for their technical advice and services; Drs. H. Levene and L. C. Dunn, Columbia University, for their patience during the course of this study and for their critical evaluation of the manuscript; Dr. M. Becker, Medical Officer, Stann Creek District, British Honduras, for permission to use the facilities of the hospital clinics in Stann Creek and Seine Bight; the Rev. G. Fairweather, who served as guide and companion while the author was in British Honduras, and to Diane Sank for assistance in the field collection of the data.

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Increased Reliability for the Determination of the Carrier State in Phenylketonuria

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THE DETECTION of heterozygotes in phenylketonuria by means of phenylalanine tolerance tests was first described by Hsia, Driscoll, Troll, and Knox (1956) and Hsia, Price, and Driscoll (1957). Later, Knox and Messinger (1958) reported that with more precise analysis, the fasting plasma phenylalanine alone was as sensitive a test for distinguishing between normal and heterozygous individuals as the phenylalanine tolerance test. Unfortunately, neither test will identify with certainty the genotype of all suspected individuals, and the problem of correctly informing anxious prospective parents that they are or are not carriers of the trait still remains.

The present study was designed to improve the information available from the tolerance test by making additional measurements after administration of phenylalanine. Since some measurements may be more important than others, combination of selected measurements would be best for differentiating between the carriers and normals. Therefore, the data presented here are treated by the method of discriminant functions (Rao, 1952) and a procedure is established whereby an individual can be assigned to one or other of the groups with less error than for any single measurement.

METHODS

The 17 heterozygotes used in this study were parents of known phenylketonuric patients and the 19 control subjects were university students with no family history of a known genetic disease. The usual dosage of 0.1 gm. L-phenylalanine per kg. of body weight was given to both heterozygotes and control subjects after an overnight fast. The amino acid was mixed with orange juice and consumed immediately after the fasting blood sample was drawn. Additional blood samples were obtained at 1, 1½, 2, 3, and 4 hours after the administration of the L-phenyalanine. When the tolerance test was performed with the DL mixture, it was fed at a level of 0.2 gm. per kg. of body weight, and the test performed in a manner similar to that for the L isomer. The first 16 normals and 14 carriers were assigned to the DL mixture. While this allotment was not strictly random, there was no known difference between the individuals assigned to the two tests. Both tolerance tests were performed on 6 heterozygotes and 4 normals, as many as could easily be convinced to submit to the venepunctures on two separate oc-

Received December 30, 1960.
Supported in part by United States Public Health grants M-2730 and M-3699.

casions. Since there was no appreciable correlation between these replicates, they have been treated as independent observations. The plasma was separated after centrifuging and stored at −10° C. until analysis. The bacterial enzyme decarboxylation method of Udenfriend and Cooper (1953) was used for the L-phenylalanine determination and L-tyrosine was measured by the nitrosonaphthol reaction of Udenfriend and Cooper (1952).

To combine the determinations into a discriminant which maximizes the intraclass correlation for the carrier and normal classes, the most convenient procedure is to assign a binary variable which takes the value 1 for carriers and 0 for normals. The usual least squares regression of this variable on the phenylalanine and tyrosine determinations as independent variates gives the coefficients of the discriminant, and the regression analysis constitutes an analysis of discrimination. This gives an optimum discriminant when the independent variables are normally distributed with the same covariance matrix in the two groups, and a generally good but not optimum discriminant when these conditions are not satisfied.

Table 1 presents the average plasma L-phenylalanine and L-tyrosine levels in normal and heterozygous subjects prior and subsequent to the ingestion of L-or DL-phenylalanine. Fasting levels of plasma phenylalanine and tyrosine fell well within the ranges reported by other workers using the same methods. Normal subjects exhibited a peak in plasma level of phenylalanine at 1 or 1½ hours after the administration of L-phenylalanine, and at 2 hours after the administration of the DL mixture, after which the levels gradually approached the normal

TABLE 1. PLASMA PHENYLALANINE AND TYROSINE LEVELS IN HETEROZYGOUS AND IN NORMAL SUBJECTS AFTER ADMINISTRATION OF PHENYLALANINE

	I L-phenylalanine (0.1 gm./kg.)		II DL-phenylalanine (0.2 gm./kg.)	
	Normal (7)	Carrier (9)	Normal (16)	Carrier (14)
	plasma L-phenylalanine (mg./100 ml.)		plasma L-phenylalanine (mg./100 ml.)	
Fasting	1.0 ± 0.2*	1.5 ± 0.2	1.0 ± 0.1	1.4 ± 0.1
1 hour	8.6 ± 1.1	12.2 ± 2.2	4.6 ± 0.4	5.7 ± 0.4
1½ hours	8.3 ± 0.9	14.5 ± 1.5	6.3 ± 0.4	7.7 ± 0.5
2 hours	7.4 ± 0.8	13.2 ± 0.6	7.3 ± 0.4	8.9 ± 0.6
3 hours	5.1 ± 0.5	10.8 ± 1.2	6.5 ± 0.4	10.2 ± 0.7
4 hours	3.4 ± 0.5	8.6 ± 1.1	5.7 ± 0.3	9.1 ± 0.7
	plasma L tyrosine (mg./100 ml.)		plasma L tyrosine (mg./100 ml.)	
Fasting	1.5 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.5 ± 0.1
1 hour	3.0 ± 0.2	1.8 ± 0.2	3.1 ± 0.2	2.1 ± 0.2
1½ hours	3.3 ± 0.2	1.8 ± 0.2	3.5 ± 0.7	2.0 ± 0.2
2 hours	3.7 ± 0.3	2.1 ± 0.3	4.1 ± 0.2	2.5 ± 0.2
3 hours	3.8 ± 0.4	2.2 ± 0.2	4.3 ± 0.3	2.8 ± 0.2
4 hours	3.8 ± 0.4	2.4 ± 0.3	4.2 ± 0.1	2.7 ± 0.2

* ± = standard error

Figures in parentheses represent number of individuals.

TABLE 2. TEST OF HETEROGENEITY OF DISCRIMINANTS BASED ON DL- AND L-PHENYLALANINE TESTS, CONVERTED TO COMMON LOGARITHMS

Source	Sum of Squares due to discrimination (S.S.)	Degrees of freedom (d.f.)	Multiple correlation coefficient squared (R^2)
DL discriminant	6.337	12	.849
L discriminant	3.881	12	.986
Discriminants (pooled)	9.500	12	.833
Homogeneity	0.718	12	—

Error variance = 0.059, d.f. = 20

$F_{12,\ 20} = 1.01$, n.s.

value in the manner of the usual tolerance curve. The plasma tyrosine levels were also increased but to a lesser extent and they remained high for a longer period. The peak of plasma phenylalanine values was higher and attained later in heterozygous subjects than in normal. In contrast, the plasma tyrosine levels were found to be consistently lower than those of the normal individuals.

The plasma amino acid measurements were converted to common logarithms to stabilize the standard deviations which otherwise tend to be proportional to the means. The error variances for the two tests were found to be homogeneous and so were pooled. Table 2 shows that in this sample the tolerance test using the L-phenylalanine is better than that of the DL mixture for distinguishing between the normal individuals and the heterozygotes. However, there was no statistically significant difference between the two discriminants and they were therefore pooled for further calculations.

Table 3 gives the analysis of the pooled discriminant. The fasting phenylalanine level is the best single discriminator, followed by the 3 hour and 4 hour levels and the 1½ hour tyrosine level, in that order. When all measurements are combined into a single discriminant, they account for 83.3 percent of the total variation, which is a significant improvement over the 65.6 percent due to the fasting phenylalanine level alone. However, the tyrosine determinations do not contribute significantly to the discriminant, which still accounts for 79.4 percent of the variation when the tyrosine test is omitted. Even if all the tyrosine values are combined, the discriminant is less satisfactory than the fasting phenylalanine level alone, accounting for only 56.2 percent of the variation.

The best discriminant therefore is based on the phenylalanine measurements. The one hour and four hour values are least critical, and may be omitted without reducing the discrimination significantly. To this simplified discriminant, which accounts for 77.7 percent of the variation, the most important contributions are made by the fasting, 3 hour, 2 hour, and 1½ hour phenylalanine levels, in that order. The common logarithms of these values are given weights of 1, .6068, - .7627, and .3578, respectively. It is interesting that the 2 hour value is given a significantly negative weight, showing that the area under the tolerance curve is not the best discriminant.

The discriminant score of the normal group ranges from 1.221 to 1.407 with a mean of 1.315 and a standard deviation of .0536. The carriers range from

TABLE 3. CONTRIBUTIONS OF THE MEASUREMENTS OF PHENYLALANINE AND TYROSINE AT DIFFERENT TIMES TO THE DISCRIMINATION OF CARRIERS

	d.f.	S.S. due to discrimination	R^2	Significance
All measurements	12	9.500	0.833	fasting phenylalanine†
				2 hour phenylalanine*
X_1 to X_7	6	9.059	0.794	fasting phenylalanine†
			3.9% due to X_8 to X_{13}	1½ hr. phenylalanine*
				2 hr. phenylalanine†
X_1, X_2, X_4, X_5, X_6	4	8.866	0.777	fasting phenylalanine†
			1.7% due to X_3, X_7	1½ hr. phenylalanine*
				2 hr. phenylalanine†
				3 hr. phenylalanine†
X_1, X_2, X_6	2	7.498	0.657	fasting phenylalanine†
			12.0% due to X_4, X_6	
X_1, X_8 to X_{13}	6	6.412	0.562	1½ hr. tyrosine*
				2 hr. tyrosine*
X_1, X_2	1	7.477	.656	fasting phenylalanine†

X_1 = Normal (0), Carrier (1)
X_2 = Fasting level phenylalanine
X_3 = 1 hour level phenylalanine
X_4 = 1½ hr. " "
X_5 = 2 hour " "
X_6 = 3 hour " "
X_7 = 4 hour " "
X_8 = Fasting level tyrosine
X_9 = 1 hour " "
X_{10} = 1½ hr. " "
X_{11} = 2 hr. " "
X_{12} = 3 hr. " "
X_{13} = 4 hr. " "

* $P < .05$
† $P < .01$

TABLE 4. ESTIMATES, $D/\bar{s}$, OF THE POWERS OF VARIOUS METHODS OF DISCRIMINATING BETWEEN HETEROZYGOTES AND HOMOZYGOUS NORMALS

Measurement	This report	Renwick *et al.* (1960)	Hsia *et al.* (1956, 1957)	Knox and Messinger (1958)
Fasting phenylalanine	2.7	1.8	1.2	1.9
1 hour "	0.9	1.2	3.2	—
1½ hour "	1.2	—	—	—
2 hour "	1.3	2.4	2.7	—
3 hour "	2.2	—	—	—
4 hour "	2.1	2.7	2.5	—
Fasting, 1½, 2, 3 hours phenylalarine	3.6	—	—	—
Fasting tyrosine	0.3	—	—	—
1 hour "	1.6	—	—	—
1½ hour "	2.1	—	—	—
2 hour "	1.8	—	—	—
3 hour "	1.7	—	—	—
4 hour "	1.5	—	—	—

TABLE 5. SCORES FOR INDIVIDUALS TESTED TWICE

		Test	
	Subject	L-phenylalanine	DL-phenylalanine
Heterozygotes	W.M.	1.6028	1.5419
	S.M.	1.5653	1.4422
	C.H.	1.6530	1.6130
	J.H.	1.4990	1.6696
	R.L.	1.4824	1.5320
	M.L.	1.4445	1.5310
Normals	S.S.	1.3010	1.3002
	M.E.	1.2256	1.3594
	R.P.	1.3550	1.3384
	N.C.	1.2400	1.2590

1.424 to 1.670 with a mean of 1.560 and a standard deviation of .0833. Each of the means is based on 23 observations and each of the standard deviations on 20 degrees of freedom. The discriminating power is measured by $D/\bar{S} = 3.6$ (Penrose 1951) where D is the difference between the means and $\bar{S}$ is the average of the two standard deviations. This compares with 2.7 for the fasting level alone and 3.3 or 3.2 for the best discriminants proposed by Renwick, Lawler and Cowie (1960) and by Hsia, Driscoll, Troll and Knox (1956), and Hsia, Price and Driscoll (1957). Using the antimode as the critical value to discriminate between carriers and normals, the classification error is less than four per cent for our discriminant, but at least nine percent for the fasting level alone. The practice of choosing among several variates the one with the greatest value of $D/\bar{S}$ leads to a spuriously high estimate of the discriminating power, and therefore the classification error associated with the best discriminant of Renwick, Lawler and Cowie (1960) cannot be evaluated. The conclusion that a more complete discriminant would be better than any single measurement was anticipated by Renwick, Lawler and Cowie (1960) who stated that "There are indications that, in spite of the marked correlations among the 0, 1, 2, and 4 hour phenylalanine levels, a more time-consuming criterion based on all four of the phenylalanine levels would have been slightly more powerful". The question arises whether our discriminant would be best in the hands of other investigators. Renwick, Lawler and Cowie (1960) found the four hour test to be superior, Hsia, Driscoll, Troll and Knox (1956) and Hsia, Price and Driscoll (1957) obtained most satisfactory results with a one hour test, while we find the fasting level to be the best single discriminator, and all of these single determinations are significantly inferior to the combined discriminator in our material. Hsia and Steinberg (1960) used the sum of the one and two hour phenylalanine levels, which Steinberg (personal communication) had earlier concluded to be as satisfactory as a discriminant using all of the data. Some of the discordances among studies may be nonsignificant, but others may be be due to the conduct of the test, including duration of fast and details of the phenylalanine assay. Until these differences are resolved, the best procedure is for each investigator to determine the discriminant which minimizes misclassification in his material.

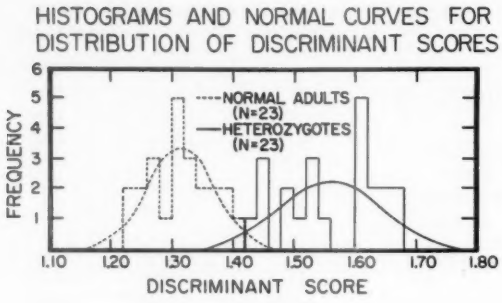

FIG. 1

Several methods may be used to reduce the classification error, including increased precision of assay, multiple determinations, taking account of the prior odds of the two genotypes, and putting borderline values of the discriminant into a doubtful group for retesting or exclusion from the analysis. For any case where the correct specification of the genotype is critical, but there is overlap between carriers and normals for any single measurement, the increased precision which the discriminant gives will amply justify the slight arithmetical labor involved. Fig. 1 gives the histogram of the scores between carriers and normals, which in our sample do not overlap. The distribution of carriers is not only significantly more variable than the normals, but shows two sharp peaks which, if supported by more observations, would suggest either multiple alleles at the phenylketonuria locus or a major modifier. However, other studies of carriers did not indicate heterogeneity, and the standard deviation of replicate scores on the six individuals tested twice is .0812, or almost as great as for different individuals. Thus heterogeneity of phenylketonuria carriers is unlikely to be detected by present methods.

SUMMARY

The best and most economical discriminant for phenylketonuric carriers is found to be

$$D = \phi_0 + .3578\ \phi_{1.5} - .7626\ \phi_2 + .6068\ \phi_3,$$

where ϕ_0 is the common logarithm of the fasting plasma phenylalanine value in mgm/1000 ml. plasma and $\phi_{1.5}$, ϕ_2, and ϕ_3 are the logarithmic levels at 1½, 2, and 3 hours respectively, after the administration of phenylalanine. Tyrosine determinations and phenylalanine levels at one hour and four hours do not contribute significantly to this discriminant. The D value for normal subjects lies between 1.221 to 1.407, whereas for the carriers it lies between 1.424 to 1.670. If values less than 1.415 are classified as normal homozygotes, and larger values are classified as heterozygous carriers, the classification error is calculated to be less than four per cent, compared with at least nine per cent for the best single measurement.

ACKNOWLEDGEMENT

We are indebted to Mrs. Nancy Jones who aided greatly in some of the tabulations. The Computer, IBM 650, was made available to us through the courtesy of the Numerical Analysis Laboratory, University of Wisconsin, Madison, Wisconsin.

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The Frequency of Elevations in the A_2 and Fetal Hemoglobin Fractions in the Natives of Liberia and Adjacent Regions, with Data on Haptoglobin and Transferrin Types[1]

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BETWEEN the years 1955 and 1959, a total of 2962 whole blood specimens were obtained by venipuncture from members of the tribes of Liberia and adjacent regions. Hemoglobin levels were determined with a Beckman spectrophotometer for all except the relatively few specimens in which, by accident or design, the blood was clotted. All specimens were examined with respect to hemoglobin types by means of paper electrophoresis. The proportion of hemoglobin resistant to denaturation by alkali was ascertained in 2088 specimens. This determination can be performed on both clotted and unclotted specimens. In addition, solubility, hemoglobin A_2 levels, and other studies were carried out where indicated and when sufficient hemoglobin was available. Finally, subsamples were studied with respect to blood types and haptoglobin and transferrin types.

Previous reports on portions of this material have established that the tribes of southeastern Liberia and the adjacent portions of the Ivory Coast have a remarkably low frequency of the genes responsible for hemoglobins S and C, by comparison with most of the other tribes of West Africa (Neel *et al.*, 1956; Livingstone, 1958a, 1960). Although in the northwest of Liberia 15 to 20 per cent of individuals are heterozygous for the sickle cell gene, at the other (southeastern) end of the country, a distance of some 350 miles, less than one per cent of the tribesmen possess the gene. The gene frequency remains low in the adjacent Ivory Coast but then rises sharply as one approaches Ghana or proceeds northward. Studies of the haptoglobins and the ABO; MN, U, and He; Rh; Fy^a; K; P and Tj^a; Jk^a; and Di^a blood groups revealed no convincing evidence for the occurrence of other, parallel clines, although the data were sug-

Received December 19, 1960.

[1] These studies were supported in part by a grant from the Rockefeller Foundation, for which grateful acknowledgment is made. It is also a pleasure to acknowledge the generous hospitality of the Liberian Institute of Tropical Medicine.

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gestive with respect to the haptoglobins and He and U blood groups (Sutton *et al.*, 1959; Livingstone *et al*, 1960). The anthropological implications of this have been discussed in detail elsewhere (Livingstone, 1958b, 1960; Rucknagel and Neel, in press). In brief, it appears that the sickle cell gene is only now diffusing into many of the tribes inhabiting this tropical rain-forest country.

In 1958 we encountered in southeastern Liberia two children who from the clinical standpoint met the criteria for the diagnosis of thalassemia major, although family studies in the case of one child raised the possibility of a somewhat different genetic basis for the disease than in the usual case (*i.e.*, combination of a thalassemia and a "high fetal" gene) (Olesen *et al.*, 1959). Up to that time, only one other child with apparent thalassemia major had been recognized south of the Sahara (Stijns, quoted in Vandepitte, 1959). This observation directed our attention to the possible occurrence in this region of genes resulting in the thalassemia phenotype. It will be the purpose of the present communication to summarize the evidence on this point, none of which has been previously presented in any detail. It will be shown that this evidence points to the occurrence of genes of the thalassemia complex throughout Liberia, with possibly a higher frequency of thalassemia in the tribes with higher frequencies of the sickle cell gene, rather than in the tribes with lower frequencies. Inasmuch as the thalassemia phenotype is frequently due to mutation at the locus[6] responsible for hemglobins S and C (Neel, 1958; Ceppellini, 1959), this finding provides additional evidence regarding the lack of genetic equilibrium at this locus in this region, a disequilibrium probably to be attributed to the relatively recent introduction into this area of both the $Hb_\beta{}^S$ and $Hb_\beta{}^C$ genes and the selective agent (falciparum malaria) to which these genes appear to confer a relative resistance.

DATA

The precise diagnosis of thalassemia minor under field conditions presents well recognized difficulties. Ideally the diagnosis rests on microcytosis with a "compensatory" polycythemia, increased erythrocyte resistance to hypotonic saline, and characteristic morphological abnormalities in the form of ovalocytes and target cells. In some instances serum iron concentration determinations, a therapeutic test with iron, or family studies may be necessary to differentiate between thalassemia and a nutritional or blood loss anemia. Recently an elevation in the A_2 component of hemoglobin has been very frequently encountered in the thalassemia minor seen in individuals of Italian and Greek origin (Kunkel and Wallenius, 1955; Kunkel *et al.*, 1957; Gerald and Diamond, 1958). Unfortunately, this useful diagnostic handle is encountered in only about half of American Negroes with thalassemia minor, so that it can be used as only one of several diagnostic criteria (Cohen *et al.*, 1959).

The present data are the by-product of a study designed primarily to deter-

[6] This locus is currently designated the Hb_β locus because it controls the β polypeptide of hemoglobin. The sickle cell gene is designated as $Hb_\beta{}^S$ and the hemoglobin C gene as $Hb_\beta{}^C$.

TABLE 1. THE HEMOGLOBIN PATTERNS ENCOUNTERED IN THE TRIBES OF LIBERIA AND SOME ADJACENT REGIONS

Tribe	Hemoglobin Pattern								Gene Frequencies**	
	A	AS	AC	C	S	AK	AN	Total	$Hb\beta^S$	$Hb\beta^C$
Northwestern Liberia:										
Kissi	113	28						141	.099	—
Gbandi	133	19	1	1	1			155	.065	.006
Loma	281	41	5		1		2	330	.064	.008
Belle	11	2						13	.077	—
Mende	50	11						61	.090	—
Vai	53	4						57	.035	—
Gola	139	16	1		1		1	158	.054	.003
Dei	37							37	—	—
Kpelle	349	37	6		1	1	5	399	.048	.008
Total	1166	158	13	1	4	1	8	1351	.060	.005
Central Liberia:										
Bassa	258	23			1	1		283	.044	—
Mano	111	¶3	1				1	116	.013	.004
Gio	101	3					1	105	.014	—
Total	470	29	1		1	1	2	504	.031	.001
Southeastern Liberia:										
Krahn	88							88	—	—
Kru	139	1						140	.004	—
Webbo	179	1						180	.003	—
Grebo	103	1						104	.005	—
Total	509	3						512	.003	—
Other Liberians:										
Americo-Liberians	11	5						16	.156	—
Mandingo	37	16	2					55	.145	.018
Miscellaneous*	11	3	1					15	.100	.033
Total	59	23	3					86	.134	.017
Ivory Coast:										
Kru	36							36	—	—
Bakwi-Oubi-Ouanbi	9	1	1					11	.045	.045
Wobe-Guere	50		1					51	—	.010
Bete-Dida-Godye-Neyo	32	1	1					34	.015	.015
Lagoon peoples†	43	2	1					46	.022	.011
Agni-Baule	31	3	1					35	.043	.014
Mandingo-Dyula	28	2						30	.033	—
Total	229	9	5					243	.019	.010
Upper Volta:										
Mossi	54	6	9	2				71	.042	.077
Miscellaneous‡	53	7	10		1			71	.056	.070
Dahomey-Togoland Tribes§	28	5	1					34	.074	.015
French West Africa Tribes‖	63	8						71	.056	—
Fulani Group	13	3	3					19	.079	.079

TABLE 1.—*Continued*

* Miscellaneous includes Bullom, Temne, Ga, Fanti, "Creole," and West Indies and American Negroes.

† Lagoon peoples include Abey, Attie, Abure, Ebrie, Avikam, Abidji, Adjukru, and Gwa.

‡ Miscellaneous includes Boboting, Senufo, Gurunsi, Djerma, Bobo, Diagouba, Kulango, Bisa, Vike, Dogon, Lobe, Birifor, Samogo, and Gurma.

§ Includes Tem, Losso, Bassari, Yoruba, Dahomey, "Togolaise," Petah, Mina, Bariba, and Fou.

‖ Includes Arabe, Yalunka, Kpelle, Rilsienne, Susu, Saracole, Yakoba, Benta, Guro, Hausa, Roumateh, Gambois, Loma, Wolof, Gabonnaise, Khassonke, Carteron, Serer, Ronanko, Tukulor, Gamo, and "Metis."

¶ On the basis of solubility studies, one of these specimens appears to be a D trait.

** Computed on the assumption that the individuals with all-S or all-C patterns are really heterozygotes.

mine the frequency of hemoglobin, blood group, and serum protein types in West African populations. They leave much to be desired in the precise diagnosis of thalassemia minor and related conditions and should be regarded as most preliminary in nature, to be followed in due time by a proper survey. The available data concerning thalassemia will be presented under three headings, followed by data on the haptoglobins and transferrins.

1) *The findings on hemoglobin electrophoresis.*—Electrophoresis was routinely performed on paper in a horizontal type apparatus at pH 8.6 with veronal buffer. The findings are summarized in table 1. This tabulation includes the 902 determinations published by Neel *et al.* (1956).

The tribes have been grouped under three headings, based primarily on geography, but also in part on language. Thus, all the tribes of southeastern Liberia speak languages belonging to the Kwa family. The central group includes one tribe (Bassa) which speaks a Kwa language and two (Mano and Gio) which speak a Mande language. The tribes of northwestern Liberia are a mixed group linguistically, including Mande-speakers (Gbandi, Loma, Mende, Vai, and Kpelle), Kwa speakers (Belle and Dei) and West Atlantic speakers (Kissi and Gola). The designation of tribes is that followed in other publications from our group and is in general in keeping with that of Murdock (1959), with the following exceptions: 1) Webbos have been distinguished from Grebos, 2) the Sapo are included with the Krahn, 3) the Mano are distinguished from the Guere, and 4) the Belle are distinguished from the Gbandi.

The previously reported cline in the frequency of the $Hb_\beta{}^S$ gene is confirmed by these additional data. There is a similar but less striking cline with respect to the $Hb_\beta{}^C$ gene. Hemoglobin D has the same electrophoretic mobility as S on paper electrophoresis at pH 8.6 but the solubility of the ferrohemoglobin is quite different (Itano, 1951, 1953). During the early years of the study 109 specimens which appeared to be AS by paper electrophoresis were subjected to solubility studies. Only one specimen had a solubility which clearly indicated that hemoglobin S was not present—this is presumed to have been AD. It may thus be concluded that the unrecognized occurrence of hemoglobin D does not significantly influence the findings regarding hemoglobin S.

TABLE 2. HEMATOLOGICAL FINDINGS IN INDIVIDUALS WITH ALL-S OR ALL-C PATTERNS ON PAPER ELECTROPHORESIS

Specimen number	Tribe	Type	Sex	Age	Per cent fetal hemoglobin	Total hemoglobin (gms %)
L0300	Bassa	S	M	adult	6.6	11.5
					5.6	15.2*
L0821	Kpelle	S	M	adult	1.9	11.6
L1413	Gbandi	S	M	adult	5.0	11.6
L2106	Loma	S	F	adult	<2.0	8.0†
S0236	Gola	S	M	50	<2.0	10.4
I0117	Senufo	S	F	19	23.2	8.4
L0388	Gbandi	C	M	A	3.6	12.8
I0278	Mossi	C	M	22	—	—
I0324	Mossi	C	M	32	<2.0	12.5

* Repeat study 8 months later.
† Pregnant at time of study.

Of especial interest in the present context is the occurrence of individuals whose hemoglobin upon electrophoresis appears to be entirely of type S or C. The findings are given in table 2. These individuals were all adults. Their hemoglobin levels are in the low normal range for adult Africans. Thus, the four all-S males averaged 11.7 gms. per cent of hemoglobin, whereas the average of 1041 hemoglobin determinations in *random* Liberian males was 13.0 ± 0.06 gms. per cent, with a standard deviation of 1.9 gms. per cent. Similarly, the two all-S females averaged 8.2 gms. per cent hemoglobin, whereas in 407 randomly selected females, the mean value was 11.6 ± 0.09 gms. per cent, with a standard deviation of 1.9 gms. per cent. As noted, one of these women was pregnant; pregnant women in Liberia were found to average 9.9 ± 0.19 gms. per cent of hemoglobin, with a standard deviation of 2.0 gms. per cent (Livingstone *et al.*, 1960). Fetal hemoglobin levels (see below) were mildly elevated in two persons and markedly in one. With respect to the all-S patterns, both the survival of these individuals to maturity and the hemoglobin levels are incompatible with the well established facts concerning sickle cell anemia in Africa (Lambotte-Legrand, 1951, 1955; Foy *et al.*, 1951; Vandepitte, 1954).

It is well known that an electrophoretic pattern indistinguishable from that of sickle cell anemia may result from the combination of a single $Hb_\beta{}^S$ gene with a gene of the thalassemia complex. The phenotype which results from this combination exhibits a considerable range, some individuals exhibiting a hemolytic anemia comparable in severity to that of classical sickle cell anemia, other individuals showing a much more benign picture (review in Zuelzer *et al.*, 1956). It is our belief that the all-S patterns encountered in the present study, and probably some of the all-C patterns, almost certainly represent sickle cell-thalassemia or hemoglobin C-thalassemia heterozygotes. Critical evidence would come from family studies, thus far lacking. If this assumption is correct, then a *minimum* estimate of the frequency of the thalassemia gene (or genes) may be obtained from the ratio of (all-S patterns)/(AS + all-S patterns). The estimate is *minimum* because 1) the double heterozygotes may

be assumed to have a significantly increased death rate, and 2) double heterozygotes do not always exhibit the all-S pattern; in some instances the electrophoretic pattern is that of the simple sickle cell trait (cf. Cohen *et al.*, 1959). With this approach, a minimum estimate of the frequency of the thalassemia gene in the northwestern tribes is 2.5 per cent, while for the central it is 3.3 per cent. This would correspond to phenotype frequencies for thalassemia minor (the heterozygote) of approximately 5.0 and 6.6 per cent respectively. There is not enough sickling in the southeastern tribes to permit a similar estimate. The use of the much scantier hemoglobin C data in the same fashion is rendered unreliable by the poor information of the survival of the homozygote for the $Hb_\beta{}^C$ gene under African conditions. However, gene frequency considerations render it improbable that any homozygote for the $Hb_\beta{}^C$ gene would be encountered in a survey of this magnitude. If we were to assume that these all-C patterns result from simultaneous heterozygosity for the $Hb_\beta{}^C$ and thalassemia genes, then by the same process of reasoning the frequency of thalassemia minor would be estimated on the basis of a very small sample indeed at 14.3 per cent in the northwestern tribes. A similar line of reasoning points to an even greater frequency of the thalassemia complex in the Upper Volta region (Rucknagel and Neel, in press), where the frequency of the $Hb_\beta{}^C$ gene is maximum (Sansarricq *et al.*, 1959).

2) *The findings on starch block electrophoresis.*—Following the demonstration of thalassemia major-like disease in two Liberian children, an effort was made to obtain a rough estimate of the frequency in Liberia of individuals in whom the proportion of hemoglobin A_2 was elevated. A total of 570 paper electrophoresis strips were scanned for evidences of an increased A_2 component. The strips were deliberately "over-read;" on this basis 46 were thought to be suggestive. In 33 of the 46 instances, there was sufficient hemoglobin left from the original specimen to permit a check by the more refined but more time consuming method of starch block electrophoresis. In 12 of these 33 specimens, the presence of increased A_2 was verified by starch block electrophoresis, any value over 3.2 per cent being considered an elevation. A summary by tribes is given in table 3. In addition to the determinations shown in the table, 17 specimens which on paper electrophoresis appeared to exhibit normal A_2 levels were selected at random and subjected to starch block electrophoresis; one of these had an A_2 elevation.

The detection of increased amounts of A_2 by the use of paper electrophoresis is unreliable, and it should be emphasized that we have not used this procedure as diagnostic but only as a clue to a possible elevation of the A_2 component. Obviously, even with the "over-reading" mentioned above some A_2 elevations were not apparent on paper and the figures obtained by the procedure described are a minimum. If we equate the presence of increased amounts of A_2 to the presence of a thalassemia gene, then a *minimum* figure for the thalassemia minor phenotype is 2.1 per cent. However, although increased A_2 levels are characteristic of the thalassemia minor encountered in individuals of Italian or Greek derivation, in our experience with thalassemia minor in Negroes less

TABLE 3. A MINIMUM ESTIMATE OF THE PROPORTION OF INDIVIDUALS WITH ELEVATED HEMOGLOBIN A_2 VALUES IN THE TRIBES OF LIBERIA*

Tribe	Number of electrophoretic patterns scanned for elevation of A_2	Number considered to exhibit elevated A_2	Number checked with starch block electrophoresis	Number checked found to have elevated A_2
Northwestern Liberia:				
Kissi	22	1	1	1
Gbandi	17	3	3	1
Loma	144	4	3	1
Belle	3	0	0	0
Mende	19	2	2	0
Vai	8	0	0	0
Gola	10	1	1	0
Kpelle	127	20	14	7
Total...	350	31	24	10 (2.9%)
Central Liberia:				
Bassa	61	8	3	0
Mano	10	0	0	0
Gio	23	3	2	2
Total...	94	11	5	2 (2.1%)
Southeastern Liberia:				
Krahn	13	0	0	0
Kru	12	0	0	0
Webbo	46	1	1	0
Grebo	28	2	2	0
Total...	99	3	3	0
Other:				
Mandingo	25	1	1	0
Americo-Liberians	2	0	0	0
	27	1	1	0
Grand Total...	570	46	33†	12

* Of the total sample of 570, there were 89 Loma, 11 Kpelle, 17 Mandingo, and 1 Gbandi known to be pregnant at the time. So far as is known, this fact should not influence the A_2 determinations.

† Of the 46 specimens thought to exhibit an elevated A_2 fraction by paper electrophoresis, in 13 instances there was insufficient blood for starch block electrophoresis. This accounts for the fact that only 33 bloods were subjected to further studies.

than half of the individuals exhibiting the cytological stigmata of thalassemia have A_2 elevations (Cohen *et al.*, 1959). This proportion may vary significantly from one locality to another, but taken at face value, an extrapolation would place a minimum figure for thalassemia in the neighborhood of four per cent.

In an earlier paper (Olesen *et al.*, 1959), on the basis of a study of only 98 specimens, a higher frequency of elevated A_2 levels was reported. The subsequent work has failed to confirm the original estimate. This is in part due to the erroneous inclusion in the earlier report of several elevations encountered in non-

random samples, but also due to an apparent lower frequency of A_2 elevations in the later specimens. Although the numbers are small and the differences not significant, the data now suggest that western and central Liberia contain if anything a higher proportion of individuals with elevated A_2 levels than eastern Liberia.

In the thalassemia minor of Italians and Greeks, the hemoglobin level averages some 2 gms. below normal (Valentine and Neel, 1948), and there may be minimal elevations in the proportion of alkaline resistant (fetal) hemoglobin (Singer *et al.*, 1951). Hemoglobin levels were determined on 7 of the 12 specimens found in the present study to exhibit A_2 elevations, six drawn from males and one from a female; the mean was 10.4 gms per cent. This is below average, but no statistical significance can be attached to the difference. None of these individuals exhibited an elevation in fetal hemoglobin. However, since the presence of large amounts of fetal hemoglobin interferes with the determination of A_2 values, an association between an A_2 elevation and a gross increase in fetal hemoglomin (see below) could not be detected.

3) *The findings on alkaline denaturation studies.*—The proportion of fetal hemoglobin present was determined in 2088 specimens by the alkali denaturation method of Singer, Chernoff, and Singer (1951). Single determinations were the rule, but some determinations, especially those yielding high values, were checked, with satisfactory reproducibility. Normally (in American Negroes and Caucasians) the proportion of fetal hemoglobin does not exceed 2 per cent. Minor elevations may be seen in adults with thalassemia minor, iron deficiency anemia, and a variety of acquired diseases. However, values over ten per cent have thus far been encountered only in thalassemia major; sickle cell anemia; combinations of a thalassemia gene with a gene responsible for hemoglobins S, C, or E; and an inherited condition characterized by a high proportion of fetal hemoglobin which may or may not be accompanied by cytological abnormalities and a mild anemia (Edington and Lehmann, 1955; Jacob and Raper, 1958; Went and MacIver, 1958; Olesen *et al.*, 1959). The precise relationship of the gene or genes responsible for this latter trait to the thalassemia gene or genes has yet to be determined by the appropriate genetic investigations, but several family studies have raised the possibility of allelism or linkage of this gene with the $Hb_\beta{}^S$ gene (Herman and Conley, 1960; Motulsky, 1960; MacIver, Went, and Irvine, in press). Since this latter gene also appears in some families to be allelic or linked with a thalassemia gene, it seems possible that allelism or linkage obtains for the "high fetal" and some thalassemia genes. The interesting theoretical implications of this finding will be discussed in detail elsewhere (Rucknagel and Neel, in press).

Table 4 summarizes the occurrence and distribution of individuals with gross elevations (greater than four per cent) of fetal hemoglobin. Although the upper limit of normal for fetal hemoglobin in this laboratory is the customary two per cent, for the purposes of this study we have set the cut-off point at four per cent in order to effect a clear distinction between meaningful variations and minor elevations of questionable significance, possibly reflecting transport and

TABLE 4. ELEVATIONS IN THE PROPORTION OF FETAL HEMOGLOBIN IN THE TRIBES OF LIBERIA AND ADJACENT AREAS. ALL TRIBAL DESIGNATIONS AS IN TABLE 1

Tribe	Proportion of hemoglobin F			Total tested
	≦4%	5–10%	≧11%	
Northwestern Liberia:				
Kissi	91			91
Gbandi	113	4		117
Loma	255			255
Belle	6			6
Mende	42			42
Vai	40			40
Gola	118	6	1	125
Dei	35	1		36
Kpelle	241			241
Total	941	11 (1.2%)	1 (0.1%)	953
Central Liberia:				
Bassa	128	3	1	132
Mano	81		1	82
Gio	62	1		63
Total	271	4 (1.4%)	2 (0.7%)	277
Southeastern Liberia:				
Krahn	49	1	1	51
Kru	106	2	1	109
Webbo	176	2	4	182
Grebo	97	1	1	99
Total	428	6 (1.4%)	7 (1.6%)	441
Other Liberians:				
Americo-Liberians	13			13
Mandingo	52			52
Miscellaneous	11			11
Total	76			76
Ivory Coast:				
Kru	36			36
Bakwi-Oubi-Ouanbi	12			12
Wobe-Guere	40			40
Bete-Dida-Godye-Neyo	26			26
Lagoon Peoples	35			35
Agni-Baule	34			34
Mandingo-Diula	12			12
Total	195			195
Upper Volta:				
Mossi	56			56
Miscellaneous	47		1*	48
Dahomey-Togoland Tribes	7			7
French West Africa Tribes	28	1		29
Fulani	6			6

* This is I0117 of Table 2.

TABLE 5. HEMATOLOGICAL FINDINGS IN THOSE INDIVIDUALS WITH GROSSLY INCREASED (>11%) FETAL HEMOGLOBIN VALUES

Specimen number	Tribe	Sex	Age	Per cent fetal hemoglobin	Total hemoglobin (gms %)
L0882	Mano	F	adult	14.8	10.5
L1037	Krahn	M	adult	13.9	15.5
L1061	Grebo	F	adult	45.0	8.2
L1083	Webbo	F	adult	29.0	9.8
				34.0	5.5*
L1166	Webbo	M	adult	30.0	9.8
				26.5	7.7
L1574	Kru	F	adult	31.0	10.7
L1634	Webbo	F	adult	32.3	8.7
L1678	Webbo	M	16	47.7	13.3
L1824	Bassa	M	adult	26.0	—†
S0046	Gola	M	adult	41.0	10.5

* Known to be pregnant at time of repeat study 18 months later.

† Specimen clotted. The recovered material yielded a value of 4.4 gms % which, while not a valid determination for computing hemoglobin levels, can be used in the determination of the proportion of fetal hemoglobin.

storage problems, laboratory error (when only a single determination is performed) or the action of factors peculiar to a tropical environment. Table 5 summarizes the hematological findings in the 10 individuals exhibiting elevations in excess of 11 per cent. For the four males on whom determinations were performed, the mean hemoglobin value is 12.0 gms. per cent, not grossly subnormal. The mean value for the five women of 9.0 gms. is relatively lower, but the numbers are inadequate for conclusions. Both normal and subnormal hemoglobin values have been reported in individuals with hereditary elevations in hemoglobin F (Edington and Lehmann, 1955; Jacob and Raper, 1958; Went and MacIver, 1958). Of the two Liberian adults with elevated fetal hemoglobin levels whom we encountered in studies on the family of a child with apparent thalassemia major, the male had a hemoglobin value of 11.2 gms. per cent and the female 8.6, but it was possible that these individuals had a thalassemia plus a "high-fetal" gene.

It will be noted that although in no instance are the numbers adequate for statistical analysis, uniformly for the all-S, the high A_2, and the high fetal specimens, the trend has been towards a subnormal hemoglobin level. This is in keeping with the results of other, more carefully controlled observations, and suggests that we are indeed detecting real entities with the present techniques.

The present data do not permit a decision concerning the genetic significance of these marked elevations in fetal hemoglobin. It is not clear to what extent these values represent the action of a single "high-fetal" gene and to what extent the result of the combination of a "high-fetal" gene with a thalassemia-type gene. Precedent exists for either interpretation. However, it will be noted that the scanty figures suggest a greater frequency of fetal values in excess of 11 per cent in southeastern than in northwestern Liberia. It will be recalled that

A_2 elevations, by contrast, were if anything less frequent in southeastern Liberia. The consequent lower likelihood of the fortuitous combination of a "high-fetal" and a "high A_2" gene in southeastern Liberia thus inclines us towards the position that the individuals with gross elevations of their fetal hemoglobin for the most part owe this to the action of a single gene.

4) *The serum haptoglobins.*—In an earlier publication we reported, on the basis of 614 determinations of serum haptoglobin types, an Hp^1 gene frequency of 0.72 in Liberia and the Ivory Coast, and suggestive evidence for a cline in the Hp^1 frequency which paralleled the $Hb_\beta{}^S$ cline (Sutton *et al.*, 1959). Because of excessive hemolysis, it was not always possible to distinguish between type 1–1 and haptoglobin-negative individuals, and all individuals not type 2–1 or 2–2 were classified as type 1–1. However, since the frequencies of the three haptoglobin types (1–1, 2–1, 2–2) as thus classified were in close agreement to those expected in Hardy-Weinberg equilibrium, it was believed that the proportion of haptoglobin-negative individuals was not high, and in any event, less than the 32 per cent reported by Allison *et al.* (1958) in southwestern Nigeria. We can now report 356 additional determinations on specimens in which hemolysis was minimal, so that the detection of haptoglobin-negatives was no problem. Techniques were as previously described. The results are summarized in table 6.

Haptoglobin-negative individuals have a frequency of 19.7 per cent in this sample. While this appears to be somewhat lower than the figure reported by Allison *et al.* (1958) or, more recently, the similar value of 32 per cent reported for 231 northern Nigerians (Habe and Fulani) by Barnicot *et al.* (1960), it is a higher value than we would have surmised from the previous studies in Liberia. Type 2M-1, as described by Giblett (1959) and Connell and Smithies (1959), was not distinguished in the previous data, but comprises 10.9 per cent of the present sample.

The much greater frequency of haptoglobin-negative individuals among West Africans than among American Negroes (Sutton *et al.*, 1959; Giblett, 1959; Giblett and Steinberg, 1960), coupled with the familial occurrence of haptoglobin-negative individuals (Harris et al., 1958a; Galatius-Jensen, 1958; Sutton *et al.*, 1959; Giblett and Steinberg, 1960), makes it very probable that both genetic and "environmental" factors are concerned in the determination of this phenotype. It is not clear whether the "environmental" factors are equally effective for all genotypes, especially in view of the fact that type 2–2 individuals characteristically have lower haptoglobin levels (as measured by ability to bind hemoglobin) than type 1–1 (Nyman, 1958). One of these "environmental" (better, non-genetic) factors in Africa may be pregnancy, since among 83 women who are included in the sample are were *known* to be pregnant, 25 (30.2 per cent) were haptoglobin negative, a departure from the remainder of the sample significant at the one per cent level. In the present sample 15.7 per cent of 172 males were haptoglobin negative, as contrasted to 23.4 per cent of 184 females. This difference is of the same relative magnitude as encountered by Barnicot *et al.* (1960). The need for specifying the age, sex, and pregnancy

TABLE 6. THE HAPTOGLOBIN TYPES OF 356 LIBERIANS

Tribe	Types of Haptoglobin										Total
	1-1		2-1		2-2		O*		2M-1		
	M	F	M	F	M	F	M	F	M	F	
Northwestern Liberia:											
Kissi	5	0	2	0	0	0	2	0	3	0	12
Gbandi	3	0	5	0	0	0	2	0	1	0	11
Loma	16	45	5	9	3	5	1	28	6	5	123
Mende	2	0	2	0	5	0	3	0	2	0	14
Vai	2	0	1	0	1	0	0	0	2	0	6
Gola	2	0	0	0	1	0	1	0	2	0	6
Kpelle	2	7	0	3	2	1	1	0	1	3	20
Total	32	52	15	12	12	6	10	28	17	8	192
Central Liberia:											
Bassa	14	0	7	0	2	0	8	0	4	0	35
Mano	1	0	1	0	1	1	0	0	0	0	4
Gio	5	0	3	0	3	0	2	0	0	1	14
Total	20	0	11	0	6	1	10	0	4	1	53
Southeastern Liberia:											
Krahn	1	1	1	0	1	0	4	1			9
Kru	0	1	0	0	0	1	1	1	2	1	7
Webbo	5	15	7	5	1	1	0	6	0	2	42
Grebo	2	9	3	5	2	0	2	2	0	1	26
Total	8	26	11	10	4	2	7	10	2	4	84
Mandingo	1	10	2	6	0	2	0	5	0	1	27
Grand Total	61	88	39	28	22	11	27	43	23	14	356

* Designates ahaptoglobinemic individuals.

status (where possible) of the individuals sampled in field studies of this system is obvious.

There is no agreement at present concerning the nature of the genetic factors influencing haptoglobin-negativity, suggestions concerning the genetic basis of the phenotype include a "silent" allele at the *Hp* locus (Harris *et al.*, 1958a; Galatius-Jensen, 1958), independently segregating genetic modifiers (Harris *et al.*, 1958a; Galatius-Jensen, 1958; Sutton *et al.*, 1959), or an allele in the *Hp* series (Hp^{2M}) which in combination with Hp^1 produces either the phenotype Hp 2M-1, haptoglobin negativity, or, possibly, phenotype Hp 2-1 (Giblett and Steinberg, 1960). Although the first of these suggestions now seems unlikely, the uncertainties and complications of the original two-allele, three-phenotype picture are at present such that attempts to calculate gene frequencies from African data present insoluble problems, and we shall refrain in this publication. This notwithstanding, one can still search for parallelisms between hapto-

globin types and Hb_β^S frequencies. A heterogeneity χ^2 for the haptoglobin phenotypes in the three tribal groupings we have employed for Liberia is non-significant ($\chi^2 = 7.87$; d.f. = 8, $0.5 > P > 0.3$). The present more limited data thus fail to bear out the earlier impression, although such departure from expectation as exists is in the same direction as observed previously.

5) *The serum transferrins.*—The serum transferrin types were determined on 333 individuals according to the methods of Smithies (1957). The results are summarized in table 7, the nomenclature following that of Giblett, Hickman, and Smithies (1959). The combined results are in general agreement with those of Barnicot *et al.* (1960) in Nigeria, and Allison and Barnicot (1960) in East Africa. Single specimens were observed of two types not encountered by these investigators, namely, a type BC and one we have termed BCD. Current genetic theory attributes each of the transferrin bands in man to the action of a different allele, so that only two bands would be expected to occur in any individual (cf. Harris *et al.*, 1958b; Smithies and Hiller, 1959), although the situation is more complex in cattle (Smithies and Hickman, 1958). The fact remains that one individual was encountered who had Beta-globulin bands in positions corresponding to transferrins B, C, and D. Unfortunately, circumstances did not permit an investigation of the iron-binding ability of the bands

TABLE 7. THE SERUM TRANSFERRIN TYPES OF 333 LIBERIANS

Tribe	Types of Transferrins				Total
	C	CD	BC	BCD	
Northwestern Liberia:					
Gbandi	11				11
Loma	118	5			123
Mende	14				14
Vai	5	1			6
Gola	5	1			6
Kpelle	19				19
Total	172	7			179
Central Liberia:					
Bassa	28	5	1		34
Mano	4				4
Gio	13	1			14
Total	45	6	1		52
Southeastern Liberia:					
Krahn	7	1			8
Kru	4	1			5
Webbo	36	2		1	39
Grebo	18	5			23
Total	65	9		1	75
Mandingo	26	1			27
Grand Total	308 (92.5%)	23 (6.9%)	1 (0.3%)	1 (0.3%)	333

or family studies. It is thus impossible to be certain that the three bands in question actually correspond to transferrins, or, if transferrins, that one band is not a modification induced by storage or contamination. Under these circumstances, it would seem advisable to consider the occurrence of such a transferrin pattern as a possibility rather than a definitive observation.

Again, evidence was sought for a cline paralleling the $Hb_\beta{}^S$ cline. A heterogenity χ^2, omitting from the calculations the two rare types, is just significant ($\chi^2 = 7.18$, d.f. $= 2$, $0.02 < P < 0.05$). Further data on this point are obviously desirable.

DISCUSSION

We have already considered the strictures to be attached to the interpretation of the data regarding the occurrence of genes of the thalassemia complex in the populations under study. Any definitive opinion must await more complete hematological studies which will include family investigations. Nevertheless, certain tentative conclusions seem justified. There is evidence for the occurrence of genes of the thalassemia complex throughout much of Liberia. The electrophoretic patterns and A_2 studies suggest that approximately four to five per cent of the population may possess a thalassemia gene; the total figure for phenotypes presenting either with thalassemia minor or with high hemoglobin F values is more like five to six per cent. The apparently greater frequency of marked hemoglobin F elevations in southeastern Liberia as contrasted to A_2 elevations in northwestern Liberia is intriguing but further data are necessary before the reciprocal relationship can be regarded as established.

It has been suggested that the thalassemia phenotype, like the sickle cell trait, confers a relative protection against falciparum malaria (Haldane, 1949; Ceppellini, 1955). The striking cline in the frequency of the sickle cell trait has been interpreted, borrowing a phrase from Fisher (1936), as "the wave of advance of an advantageous gene" (Livingstone, 1960). With the discovery of thalassemia in Liberia, the possibility arose that this might be a stationary wave, i.e., that those populations not genetically protected from falciparum malaria by the sickle cell gene might be protected by the thalassemia complex. The present data, even in their preliminary form, would seem to dispose of that possibility. There can be no doubt that the tribes of southeastern Liberia, under conditions of intense malaria pressure, are far from equilibrium at the Hb_β locus. It remains for further studies to demonstrate whether the thalassemia complex is actually *more* prevalent in the tribes of the northwest with the higher frequencies of the S and C genes, a finding which might be interpreted as the response of two genes to a common selective agent.

The data on the serum haptoglobins and transferrins have been introduced not only as a contribution to defining the genotype of the peoples under study, but especially in an effort to seek other evidences for gene diffusion similar to that shown by the $Hb_\beta{}^S$ gene. In the case of the haptoglobins, the data neither confirm nor deny the previous evidence suggestive of a parallel cline; judgement must be suspended pending clarification of the significance of the 2M-1

and haptoglobin negative types. As for the transferrins, there is borderline evidence for a cline; a doubling of the sample should suffice to settle the issue.

SUMMARY

Data from Liberia on 1) the frequency of individuals whose hemoglobin on paper electrophoresis appears all-S or all-C in type, 2) the frequency of individuals with an elevation of the A_2 component of hemoglobin, and 3) the frequency of individuals with gross elevations in the amount of fetal hemoglobin present, all combine to suggest that four to five per cent of the population may be heterozygous for a gene of the thalassemia complex.

Data are presented on the frequency of the serum haptoglobin and transferrin types, and an attempt made to relate the findings to the cline for the sickle cell gene previously demonstrated in Liberia.

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BOOK REVIEWS

Molecular Genetics and Human Disease

Edited by LYTT I. GARDNER. Introduction by Linus Pauling. Springfield, Illinois: C. C Thomas, 1961, 297 pp. $11.50.

THIS IS A BOOK to recommend to physicians and some biologists who want to know what the excitement has been about in genetics during the last few years, exclusive of the advances in microbial genetics. It is mainly the record of a symposium held at the Upstate Medical Center, Syracuse, New York, two years ago (April, 1959). The book was apparently intended to be more than a formal transcript. It has been addressed to a particular audience, and efforts were made to avoid the serious possibility that it would be hopelessly out of date before publication. For a while yet its lack of timeliness should not hinder its presentation to a primarily medical audience of a broad area related to human genetics that has recently been in ferment. Its theme is "molecular genetics as a continuum with classical genetics (represented by Crow, Newcombe, and Wallace) and with human familial disease." The area covered includes the structure of DNA and its identification as the information carrier of the gene (Crick), the genetic control of amino acid sequences in the abnormal hemoglobins (Ingram), the newer knowledge of the inborn errors of metabolism (Childs, Sidbury, Kirkman, and Kretchmer), the chromosomal abnormalities revealed by cytogenetics (Polani), and the radiation-induced increases in mutation rates (Russell).

Nearly every paper reviews the origin of the findings discussed and presents only the more established results in a relatively nontechnical manner. A glossary of some thirty terms at the end of the book defines the unavoidable jargon. The recorded discussions after each paper are frequently illuminating, since there were questions that the nonexpert reader might himself have asked and other questions which gave the speakers opportunities to outline the directions of the then current research.

The vintage of the papers can be appreciated by remembering that the 21-chromosome trisomy of mongolism was first published during the month this symposium was held. Since this important type of chromosomal abnormality was not included in the original discussions, a supplementary review of cytogenetic developments up to early 1960 has been added by the editor and his colleagues to make good this omission. But the delay in publication and the tempo of development in most of the areas have been so great that the book is already out of date for the specialists in the fields covered, hence it will be most useful to those who seek only general information.

Apparently few of the multi-authored books now popular are meant to be read from beginning to end, and such a procedure is discouraged by an inconsistent ordering of papers here. The reader who picks and chooses should be cautioned that the dust jacket lists "14 expert contributors", the "List of Contributors" includes 16, while I counted 21 authors of papers in large type. The Table of Contents includes only as "Discussants" Marks and Zinkham, who gave informative short papers on primaquine sensitivity. Other contributions of particular interest and not readily available elsewhere included an unusual humanistic introduction by Gardner, Sidbury's analysis of the "pedigree of causes" of galactosemia on the basis of galactose-1-phosphate inhibition of phosphoglucomutase, and Kirkman's review of primaquine sensitivity and his elegant comparison of the properties of the glucose-6-phosphate dehydrogenase of normal and deficient red cells in this

condition. The latter's penetrating conclusion, that "—as one more accurately measures the enzyme—it more nearly correlates with the expected genetic pattern," should be the guide for investigation of the enzyme deficiency diseases.

Kretchmer was given the awkward task of explaining that in contrast to the selected characters commonly used in experimental genetics, most of the gene expressions important in clinical medicine are being continously modified in an individual during his development and by his environment. Cogent examples were drawn from the adaptive enzymes, the hemoglobins, and the hormones.

The round table discussion on increased mutation rates in man by the audience and Crow, Gardner, Neel, Newcombe, Russell, and Wallace was a disappointment. These veterans of such discussions soberly went through their paces with little spontaneity or candor. It was left to the medical participants to raise problems of ethics and what C. P. Snow calls "statistical morality" (e.g., the problem of injury of people unidentifiable except as a statistic like the predicted 380,000 genetic deaths from the Carbon[14] released up to September, 1958).

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Medizinische Genetik: Eine Einfuhrung in ihre Grundlagen und Probleme

By Widukind Lenz. Stuttgart: Georg Thieme Verlag, 1961, 197 pp. $5.50 through International Medical Book Corporation.

Dr. Lenz's book is by far the best introduction to medical genetics for physicians and medical students which this reviewer has seen. Some excellent textbooks of human genetics are directed to nonmedical audiences. Their authors are often not familiar with the medical scene and medical readers find much material not pertinent to their interests. Other books attempting a more medical approach often have been superficial in their discussion of genetics. This book presents a critical and thorough evaluation of genetic problems as they apply to medicine. Dr. Lenz does not attempt to cover systematically all diseases with genetic etiology but limits his discussion to the role of genetic facts and concepts in the understanding of medical problems.

A general section deals with the nature of human genetics and its importance for medicine. Medical genetics has made its greatest contribution in close collaboration with biochemistry, cytology, serology and clinical medicine. The author concludes that medical genetics as an independent discipline is a sterile field.

A brief up-to-date survey of the chemical nature of the gene is followed by excellent and sophisticated discussions of simple patterns of inheritance. There are pedagogically excellent and simple explanations for handling statistical family data. Interpretations of gene actions in man are modern and thoughtful. The author's literature citations reveal a ready familiarity with the best of Anglo-Saxon, French, Scandinavian and German literature and are most helpful. In the section on chromosomal abnormalities Dr. Lenz limits his discussion to the observational aspects with some emphasis on color blindness studies and their value in identifying the source of nondisjunction. Since the usual medical reader lacks a cytogenetic background, it is hoped that in a new edition some more space will be devoted to the more basic cytogenetic implications of these recently discovered disorders.

The last section of the book deals with polygenic inheritance, twin research, and genetic

factors in common diseases in a sensible manner. The author does not hesitate to express ignorance if little is known in an area. There is a glossary of both medical and genetic terms. This reviewer will recommend the book without hesitation to German reading medical undergraduates and graduate students as the most useful current source for an understanding of the importance of genetics in medicine. It is hoped that an English translation will become available soon.

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Introduction to Quantitative Genetics

By D. S. FALCONER. New York: The Ronald Press Co., 1960, ix + 365 pp., $6.00.

THE INHERITANCE of quantitative characters is studied through the analysis of measurements, especially the mean and the variance. That part of the variance controlled by the genes determines the extent to which inherited changes can occur. It is the subject matter of this book. The writing is lucid and the material well chosen. This excellent exposition should be read by all persons in the field of genetics. Because much economically important work is in the area of quantitative genetics, an understanding of these analytical methods is of use in the practical application of genetics. On the other hand, because of the strong connection of the subject matter with the effects of artificial selection and the limited connection of this field with human genetics, medical geneticists may find only a few parts of the book to be of direct interest.

The first five chapters serve as an introduction to population genetics. The approach is restricted to systems directly applicable to work with Drosophila and mammals (e.g., polyploidy is dismissed at the outset as of no interest). The discussion presents the Hardy-Weinberg law and the amendments to it produced by migration, mutation, selection, linkage, inbreeding, and small population size. Simple algebra is employed throughout this section, without the extensive calculus which is required for a complete understanding of the subject. However, to be realistic, this is the level of mathmatical competence that can be expected of most advanced students in genetics. Thus, as an unexpected bonus, this section of the book presents what for most geneticists may be the most readily assimilable exposition of population genetics that is available.

Chapters six through ten serve to introduce the concepts specific to the study of metric characters. The reader is provided with an understanding of the methods of describing means and variances and of ascribing portions of the variance to various aspects of heredity and environment. Chapter seventeen, on transformations of scale, and chapter eighteen, on characters whose penetrance or expressivity is dependent upon physiological thresholds, should have been placed in this part of the book, where they would have contributed to the discussion which follows. In their present position, these chapters are the least effective in the book.

The eleventh through thirteenth chapters deal with laboratory studies on techniques of artificial selection, in conjunction with the section on selection of correlated characters, which seems misplaced as chapter nineteen. Artificial selection is clearly the specialty of the author and a group of his colleagues at Edinburgh and the presentation of their methodology and principles evokes admiration for the understanding that they have of their field. In contrast with this, chapters fourteen through sixteen, devoted to an analy-

sis of the effects of inbreeding and cross-breeding, are far less inspiring. Related to the lowered interest of the author in this subject, in comparison with selection, the theoretical discussion is less often and less forcefully integrated with the presentation of examples. The final (twentieth) chapter is an interesting summary of the principles of change under artificial selection under the guise of discussing natural selection. The references are almost exclusively to laboratory studies of artificial selection and almost any of the special topics could have been handled in other appropriate parts of the text.

There are few of the little defects that might annoy a reader or mar the comprehensibility of the presentation. The figures are uniformly well-drawn, most of the formulas are error-free, and there are few spelling errors. Except in the spirit of adventure, the reader is warned away from the section on linkage which begins on page 99, in which ten-fold and hundred-fold errors combine with figures that do not quite go with what is discussed in the example to give the most confused three pages in the book.

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Meticciato di Guerra e Altri Casi

By L. GEDDA, A. SERIO, and A. MERCURI. Roma: Istituto Gregorio Mendel, 1960, XII + 398 pages, Lire 10,000.

THE foreword, by Ruggles R. Gates, informs us that a "science of racial genetics is gradually emerging", and that this book will aid "in the founding of the new science." The lineaments of the alleged new science are frankly puzzling. The book under review contains an anthropometric, hematologic, and to some extent physiologic and psychometric study of 44 "war mulattoes", aged 8–12 years, children of Italian mothers and of fathers whose identity is only rarely known, but who are assumed to have been "colored soldiers" of the armies of occupation. The conclusion that these children show "morphologic heterosis" and "auxologic heterosis" is questionable. The conclusion that they possess IQ's "corresponding to at most the zone inferior to the medionormal values" is still more questionable. By an odd slip, this last conclusion is omitted in the "Summary and Conclusions" in English, French, and German, but does appear in the Italian "Riassunto e conclusioni".

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Molecular Structure and Biological Specificity

Edited by LINUS PAULING and HARVEY ITANO. Washington, D.C.: American Institute of Biological Sciences, 1960, 195 pp., $5.75.

THE present volume represents a symposium on the subject of molecular structure and biological specificity arranged by the American Institute of Biological Sciences and held in Washington, D.C. in 1955. The book first appeared in 1957 and a second printing was made in 1960. As such, the book is of historical interest as a milestone in the progress of our thinking in this rapidly developing field.

For example, the chapter on sickle cell hemoglobin by Itano was prepared before the

publication of Ingram's "fingerprinting" technique. It summarizes the very important contributions made by Pauling and his group in describing the abnormalities by electrophoretic and solubility techniques. However, of necessity, it does not describe the present thinking on the sequential abnormalities in the alpha and beta chains, and it shows how much progress has been made in such areas in the past five years.

The section on the nature and formation of antibodies by Haurowitz makes interesting reading. The author actually makes a calculation on the number of antibody molecules formed per injected antigen molecules, and the maximum time required for the formation of an antibody molecule. He then discusses whether the rate of formation is adequate to explain that all of the injected toxoid molecules act as templates.

The other sections deal with the physical chemical characteristics of the molecules, and such factors as hydrogen bonding, inter-molecular forces, and some extremely complex calculations on the London-Eisenschitz-Wang forces. It is unfortunate that the recent exciting studies of Kornberg and his associates were not as yet complete in 1955, as they would have fitted in well with the other topics in the symposia.

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The Antecedents of Man. An Introduction to the Evolution of the Primates

By W. E. Le Gros Clark. Chicago: Quadrangle Books, 1960, 374 pp. $6.00.

The author's "Early Forerunners of Man", published in 1934, was a greatly admired review of the anatomy and paleontology of the Primates. This new version adheres closely to the plan of the old, but includes knowledge of *Proconsul*, the australopithecines, and other primate fossils found since 1934. It also reflects changes in the general view of a major student of the subject, particularly his disposition to recognize connections and intergradations among the main primate groups, in place of his earlier acceptance of long independent histories for many of them. The body of the book consists of chapters which are comparative summaries respectively of the dentition, skull, limbs (much new material here), brain, senses, and digestive and reproductive systems.

All this is concisely and simply set forth, no small achievement for an authority in the middle of a large field. Le Gros Clark avoids rehashing past views and writes for the present. For example, the differences of lemurs and lorises led him, in 1934, to infer "that the Lemuriformes and Lorisiformes parted company at least before the evolutionary stage represented by the tree-shrews had been reached", i.e., doubtless before the Paleocene, but in 1960 he writes, without making obeisance to the older book, that "the derivation of the lorisiforms from the Eocene lemuriforms offers no theoretical difficulty", which is a considerable time difference and a less rigid interpretation of the possibilities. This will serve as an example both of the author's main change of view, as I see it, and of his lack of interest in having arguments with himself. This makes, like his whole style, for straightforward presentation. It will be, like its predecessor, a primary book in its field for many years.

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BOOKS RECEIVED

Natural Selection and Heredity

By P. M. SHEPPARD. New York: Harper and Brothers, 1960, 209 pp., $1.35.

This is a reprint of the book which was first published in 1958 by Hutchinson of London. It deals with some recent ideas on the mechanism of evolution in the light of modern genetics.

Developmental Genetics and Lethal Factors

By E. HADORN. Translated by Ursula Mittwoch. New York: John Wiley and Sons, Inc., 1961, 355 pp., $8.50.

This is a translation of the German edition of the book which first appeared in 1955. A review of the German edition by Curt Stern was published in this Journal 7: 439–440, 1955.

LETTER TO THE EDITOR

Incidence and Prevalence in Human Genetics

To the editor.
Dear Sir:

In the December 1960 issue of the American Journal of Human Genetics appeared two articles in which the term "incidence" was used to denote two different measures of morbidity. In the first article the term is used incorrectly and in the second correctly. This, of course, is not calamitous as long as some understanding is gained as to what is measured and how it is measured, but the misuse of this and related terms in the medical and genetic literature has in the past been, and continues to be, the rule rather than the exception. What makes such misuse particularly unfortunate is that these are not loose and general terms but well-defined descriptive ones with precise meaning.

Incidence and prevalence and their respective rates are epidemiologic terms. They are used to measure morbidity in defined populations and to compare populations differing geographically, or segments of a population, by such characteristics as age, sex, race, marital status, environmental conditions, etc. But genetic research on the population level is largely epidemiologic. Neel and Schull (1954) succinctly pointed out: "In the analysis of factors affecting both mutation and selection the population geneticist must work with population characteristics which are the stock-in-trade of the epidemiologist." These characteristics are, indeed, the basis from which selection parameters and mutation rates are estimated. The geneticist, therefore, must be prepared to use the epidemiologic method correctly and epidemiologic terminology precisely.

Shaw, Falls, and Neel, the authors of the first article, one on congenital aniridia, state on p. 393: "The civilian population of the lower peninsula of Michigan for January 1, 1959 . . . was 7,604,811, and the known living Michigan aniridia population on the same date was 118. This gives a minimum incidence figure of 1:64,448 . . ." By definition, as we shall see below, this is not incidence but prevalence.

Incidence refers to the number of *new cases* that develop during a given period per unit of population. Incidence rates are usually expressed per year per 100,000 population; thus:

$$\text{Incidence rate of illness} = \frac{\text{Number of new cases developing during 1 year}}{\text{Number of persons exposed during the year, to the risk of developing the disease}} \times 100{,}000$$

The denominator or the population "at risk" may be the entire population or, if one chooses, a specific segment with respect to age, sex, etc. within the total population. This refinement may be desirable when one is measuring the incidence rate of a disease whose age of onset covers only a limited period of years.

Prevalence denotes the total number of cases in the population, living *on a given date*. Prevalence rates are, again, usually expressed per 100,000 population; thus:

$$\text{Prevalance rate of illness} = \frac{\text{Number of cases of disease existing on a specific date}}{\text{Number of persons in the population on this date}} \times 100{,}000$$

Strictly speaking, it is more correct to call this a ratio rather than a rate. Since the time is theoretically instantaneous (although in practice it is a specified date) the number of the affected to the total number in the population at risk forms a ratio. This ratio may also be developed for subunits of the population by age, sex, etc. where indicated. The figure of 1:64,448 given by Shaw, Falls and Neel is, then, a prevalence figure. The prevalence rate of aniridia calculated from these authors' data would be 1.55 cases per 100,000 population.

In another article in the same issue, Steinberg and Brown attempt to estimate the incidence of cystic fibrosis of the pancreas in Ohio. The term is here used correctly as the number of cases among white children born alive (new cases) in Ohio during the years 1950–1953, inclusive. This is given as 1:3700. The incidence rate is calculated by the authors from the data to be 27 per 100,000. It should be noted that this rate is given without any reference to time and it may be misconstrued to mean per year, while it actually refers to the affected among newborn in the four-year period, 1950–1953. It is, therefore, birth incidence rate. The important point is that these authors were able to compare their findings with those of two other studies because their morbidity measuring yardsticks were the same —incidence of one new case per so many births.

In case this commentary may appear to be a little pedantic, may I point out that we geneticists tend to get a little annoyed when we encounter genetic terms such as penetrance, sex-limitation, familial, etc. in non-genetic publications (especially medical) used in imprecise manner to mean many things to many people. It will behove us to get used to and to employ the proper epidemiologic terms in our work and our writings since, with the close collaboration of genetics and epidemiology, they are bound to become a part of our standard nomenclature. An instructive resumé of epidemiologic concepts, methods and terminology can be found in the excellent article by Dorn (1955).

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